Assessment of Cardiovascular Risk in Women with a History of Pre-eclampsia

by

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“The important thing is not to stop questioning.
Curiosity has its own reason for existing”

- Albert Einstein

Summary

Pre-eclampsia is an important and serious condition affecting 2-8% of pregnancies worldwide and carries with it significant associated risk of morbidity and mortality for both mother and child. It is characterised by new onset hypertension after the 20th week of gestation with accompanying proteinuria. Resolution of symptoms should occur following delivery.

Several pathophysiological mechanisms are common to both pre-eclampsia and cardiovascular disease, and the link between pre-eclampsia and cardiovascular disease later in life has been established. While the underlying pathophysiological mechanisms of pre-eclampsia are complex, endothelial dysfunction is a key component. Increased arterial stiffness and hypertension have also been documented. Endothelial dysfunction has been shown to extend beyond childbirth, into the postpartum period. Studies evaluating endothelial dysfunction at even longer time-points following an affected pregnancy have produced conflicting results.

Results from biomarker studies have supported the concept of endothelial dysfunction throughout pregnancy and the postpartum period, but as more time elapses between index pregnancy and biomarker sampling, these results also vary.

Cardiac imaging and electrocardiographic studies have also contributed to knowledge about the normal physiology of pregnancy and changes which are associated with hypertensive disorders of pregnancy during pregnancy, the post-partum period and beyond.

The main focus of this thesis was to investigate the possible mechanisms behind the link between pre-eclampsia and future cardiovascular disease. The aim was to investigate women who were free from cardiovascular disease for any evidence of subclinical vascular damage long-term following a pre-eclamptic pregnancy. Overall women recruited to this study would be older than women who participated in the majority of previously published studies on this theme.

Before embarking on the investigation of subclinical vascular damage in women with a history of pre-eclampsia, a link was confirmed between a history of pre-eclampsia and cardiovascular disease up to 30 years from time of index pregnancy. This was

accomplished using record-linkage in a large Scottish cohort; the Generation Scotland Family Health Study (GS:SFHS). Following on from this, ECGs available in women with and without a remote history of pre-eclampsia in the GS:SFHS cohort were assessed for any obvious differences. There was a more leftward shift in the QRS-axis in these women and a trend towards a longer corrected QT interval (QTc) which approached statistical significance, but after adjusting for other co-variates, pre-eclampsia did not independently predict QTc.

Investigations for subclinical vascular damage were carried out by means of non-invasive vascular function studies in women recruited from three different cohorts (blood pressure clinics, GS:SFHS and the previous Proteomics in Pre-eclampsia (PIP) study of women during pregnancy). Time since index pregnancy varied between 1-30 years. Flow-mediated dilatation (FMD) was performed to assess for endothelial dysfunction, pulse wave analysis (PWA) and pulse wave velocity (PWV) assessed arterial stiffness, and carotid ultrasound was performed to establish whether there was any evidence of atherosclerosis. After adjusting for other co-variates, I was able to demonstrate the presence of endothelial dysfunction many years after pregnancy in women with a history of pre-eclampsia in comparison with those who experienced a normotensive pregnancy. There was also a significantly higher presence of carotid plaque in women with a history of pre-eclampsia.

To investigate whether the findings from the vascular study translated to findings in biomarker studies of women with a history of pre-eclampsia in comparison with controls, samples from the vascular studies cohort and from the wider GS:SFHS cohort were used. Markers of inflammation, angiogenesis, cardiac damage and collagen turnover were studied. A significantly higher vascular endothelial growth factor (VEGF) was detected in women with a history of pre-eclampsia.

Pre-eclampsia is associated with an increased risk of cardiovascular disease, and endothelial dysfunction is evident later on in life. Larger studies are required to further investigate the vascular and biomarker results, and studies including more thorough cardiac assessment (such as echocardiography) in this population should also be considered.

The studies described found no evidence of one single component to explain the relationship between pre-eclampsia and cardiovascular disease later in life. This is not unexpected as pre-eclampsia is a complex condition with multiple contributing factors and

it is likely that the increased cardiovascular risk later in life is likewise multifactorial in origin.

Table of Contents

List of Tables

List of Figures

.

Publications and Presentations

Presentations containing work undertaken for this thesis

Measurement of the corrected QT interval during pregnancy and the post-partum period. Brown CE, Friar M, Carty DM, Macfarlane PW, Delles C *(***WINNER Poster Presentation**, *Glasgow Paediatric Research Day – October 2015)*

Vascular Properties in women who had Pre-eclampsia: the Cardiovascular Consequences of Pre-eclampsia Study (COPS). Brown CE, Flynn J, Carty DM, Generation Scotland, Delles C *(Poster Presentation, British Hypertension Society - September 2015)*

Vascular Consequences Of Pre-eclampsia. Brown CE, Flynn J, Carty DM, Generation Scotland, Delles C *(Oral Presentation, European Society of Hypertension - Milan, June 2015)*

The Cardiovascular Consequences of Pre-eclampsia Study (COPS) - a summary of the vascular studies. Brown CE, Flynn J, Carty DM, Delles C *(***3rd Prize, Poster presentation**, *British Hypertension Society - September 2014)*

Cardiovascular events in women with a history of pre-eclampsia in the Generation Scotland: Scottish Family Health Study
Carty DM, Brown CE, Robinson S, Schneider M, Dominiczak A, Padmanabhan S, Delles C (*Oral presentation, British Hypertension Society, September 2014)*

The likelihood of cardiovascular events in women with a history of pre-eclampsia in the Generation Scotland: Scottish Family Health Study. Carty DM, Brown CE, Robinson S, Schneider M, Dominiczak A, Padmanabhan S, Delles C (*Oral presentation, European Society of Hypertension/International Society of Hypertension, June 2014)*

Publications related to the work undertaken in this thesis

Urinary proteomic biomarkers to predict cardiovascular events. C.E. Brown, N.S. McCarthy, A.D. Hughes, P. Sever, A. Stalmach, W. Mullen, A.F. Dominiczak, N. Sattar, H. Mischak, S. Thom, J. Mayet, A.V. Stanton, C. Delles**.** Proteomics Clin. Appl. 2015, 9, 610–617

Presentations related to the work undertaken in this thesis

Assessment of vascular phenotyping in patients at cardiovascular risk. Brown CE, Ghaus A, Moreton F, Flynn J, Currie G, Delles C (*Poster presentation, European Society of Hypertension/International Society for Hypertension - June 2014)*

Vascular Phenotyping in Patients at Cardiovascular Risk
Ghaus A, Brown CE, Currie G, Moreton F, Flynn J, Delles C
(Poster presentation, Scottish Cardiovascular Society, Aberdeen, January 2014)

QTc and QTc prolongation in the Generation Scotland Family Study. Brown CE, Hastie CE, Alghamdi J, Schulz C, Hocking LJ, Luciano M, Porteous D, Morris A, Smith BH, Generation Scotland, Dominiczak AF, Delles C, Tobias ES, Padmanabhan S (*Oral presentation, British Hypertension Society Meeting, London, September 2013)*

Predictors of QTc and QTc prolongation in the Generation Scotland Family Study. Brown CE, Hastie CE, Alghamdi J, Schulz C, Hocking LJ, Luciano M, Porteous D, Morris A, Smith BH, Generation Scotland, Dominiczak AF, Delles C, Tobias ES, Padmanabhan S (*Poster presentation European Society of Hypertension Meeting, Milan, June 2013)*

Urinary Proteomics and cardiovascular events. CE Brown, H Mischak, A Albalat, W Mullen, N Sattar, N S McCarthy, A D Hughes, S Thom, J Mayet, A Stanton, P Sever, A F Dominiczak, C Delles (**WINNER of Sir Jack Ledingham Prize** for Poster presentation, *British Hypertension Society Meeting, London, September 2013)*

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On a more personal note I would like to acknowledge the great example set by my father, Dr John H Brown, which has helped shape my career choices in life. I would like to thank him and my mother, Arlene, for their constant support and assistance with childcare. This work would also not have been possible without the help of my amazing husband Craig who has been my rock throughout. Finally, I would like to dedicate this thesis to my wonderful son, Struan.

Author's Declaration

The experimental design of the work presented in this thesis was that of Professor Christian Delles, Dr David Carty and the author.

All experimental work and analyses presented in this thesis were carried out by the author with the exception of some of the vascular studies in chapter 5 and the biomarker studies in chapter 6. Research nurse Joanne Flynn carried out some of the vascular studies in chapter 5 and several people were involved in carrying out the biomarker studies. These included Randox Investigator Platform testing carried out by Dr Anne Marie Jennings (Randox Laboratories, Crumlin, UK), ELISA studies carried out by Dr Susana Ravassa (University of Pamplona, Navarra, Spain), and Luminex Magpix testing carried out by Ms Liliya Sharafetdinova, (ICAMS, BHF GCRC). Urinary proteomic studies were also carried out by Ms Sharafetdinova and were overseen by Dr William Mullen, (ICAMS Proteomics Laboratory).

Processing of COPS study samples was performed by Elaine Butler (ICAMS). Mr Scott Robinson assisted with the preparation of the dataset used in chapter 3.

This thesis has been composed by the author as a record of work performed during my time as a Clinical Research Fellow at the Institute of Cardiovascular and Medical Sciences, University of Glasgow. It has not previously been submitted for a higher degree.

Catriona Brown
2017

List of abbreviations

AIx	Augmentation index
ASE	American Society of Echocardiography
BHF	British Heart Foundation
BMI	Body mass index
BP	Blood pressure
CAD	Coronary artery disease
CCA	Common carotid artery
CE	Capillary electrophoresis
CHI	Community Health Index
CI	Confidence interval
cIMT	Carotid intima-media thickness
CKD	Chronic kidney disease
COPS	Cardiovascular Consequences of Pre-eclampsia Study
CRP	C-reactive protein
CS	Caesarean section
CV	Coefficient of variation
CVA	Cerebrovascular accident
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DICOM	Digital Imaging and Communications in Medicine
DNA	Deoxyribonucleic acid
ESH	European Society of Hypertension
GCRC	Glasgow Cardiovascular Research Centre

GS	Generation Scotland
GS:SFHS	Generation Scotland: Scottish Family Health Study
HDL	High density lipoprotein
HELLP	Haemolysis, elevated liver enzymes, low platelets
HR	Hazard ratio
HRT	Hormone replacement therapy
ICA	Internal carotid artery
ICAM	Intercellular adhesion molecule
ICD	International Classification of Disease
IFN	Interferon
IHD	Ischaemic heart disease
IL	Interleukin
IMT	Intima-media thickness
IQR	Interquartile range
ISD	Information Services Division
ISSHP	International Society for the Study of Hypertension in Pregnancy
IUGR	Intra-uterine growth restriction
LDL	Low density lipoprotein
MAP	Mean arterial pressure
MS	Mass spectrometry
NO	Nitric oxide
OR	Odds ratio
PE	Pre-eclampsia
PIP	Proteomics in Pre-eclampsia

PlGF	Placental growth factor
PP	Pulse pressure
PWA	Pulse wave analysis
PWV	Pulse wave velocity
QTc	Corrected QT interval
RAAS	Renin-angiotensin aldosterone system
REC	Research Ethics committee
ROS	Reactive oxygen species
SBP	Systolic blood pressure
SD	Standard deviation
sFlt-1	Soluble FMS-like tyrosine kinase 1
SMR	Scottish Morbidity Record
SPSS	Statistical package for the Social Sciences
TC	Total cholesterol
TG	Triglycerides
TGF	Transforming growth factor
TIA	Transient ischaemic attack
TNF	Tumour necrosis factor
VCAM	Vascular cell adhesion molecule
VEGF	Vascular endothelial growth factor

1. Introduction

1.1 Introduction

Pre-eclampsia affects 2-8% of all pregnancies and is a leading cause of both maternal (1) and fetal (2) morbidity and mortality with up to 40,000 women world-wide dying from the condition annually (3;4). Nearly 40% of births which are delivered before 35 weeks of gestation are due to pre-eclampsia (5).

A relationship between pre-eclampsia and future maternal cardiovascular risk has been widely established (6), however the underlying mechanisms of this relationship remain unclear. In an attempt to unravel possible contributing factors to the risk of cardiovascular disease years after a pregnancy complicated by pre-eclampsia, this chapter will first consider the changes the cardiovascular system experiences during pregnancy. It is then important to explore what is different in pre-eclampsia, and the pathophysiology of pre-eclampsia. Following on from this one must consider what is currently known about the nature of the cardiovascular risk in women with a history of pre-eclampsia before finally examining the possible methods by which this could be further investigated, from the cellular and molecular level through to the clinical and vascular level.

1.1.1 The cardiovascular system in pregnancy

In normal human pregnancy there is vasodilation of the systemic vasculature which begins very early at around 5 weeks gestation (7). There is a decrease in peripheral vascular resistance which starts during the first trimester and continues into the second trimester. It then increases towards the end of pregnancy. In the post-partum period systemic vascular resistance has increased such that a few weeks after delivery the body has adjusted back to its pre-pregnant state (7;8). Blood volume increases throughout pregnancy (7) and red cell production is also increased but not proportionally as high as plasma volume therefore haemoglobin levels drop overall, causing the physiological anaemia of pregnancy.

Cardiac output increases reaching its peak between the second trimester and term (9). It is 15 % higher in twin pregnancy than singleton pregnancy (7). Heart rate increases (9) and

stroke volume increases until the end of the second trimester when it may stay constant or decrease (7). Pregnancy is associated with cardiac hypertrophy which is physiological and generally returns to normal by 1 year (9).

Systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP) all decrease during pregnancy, dropping to their lowest point mid-trimester (7;10). Blood pressure then rises again until delivery. One possible reason for this mid-trimester drop is thought to be the development of the utero-placental system, which is a low-resistance system (11).

A recent study by Nama et al challenged the concept of the mid-trimester drop in blood pressure in normal pregnancy (12). In a prospective study of 255 primigravid women who were normotensive at booking and had blood pressure measured at four time-points during gestation, SBP was found to increase throughout pregnancy. DBP also increased after an initial dip at 22 weeks gestation. Another study performed shortly after by Grindheim et al (11) with 57 normotensive women measured heart rate and blood pressure at 4 time points during pregnancy and at 6 months post-partum. There was a significant mid-trimester decrease in SBP, DBP and MAP confirming the findings of previous studies (13;14).

1.1.2 **Hypertensive disorders of pregnancy**

1.1.2.1 ***Diagnostic criteria***

Throughout the period of research resulting in the studies described in this thesis there have been updates both in the International Society for the Study of Hypertension in Pregnancy (ISSHP) guidelines (15;16) and in the guidelines from the American College of Obstetricians and Gynaecologists (17). A breakdown of criteria from ISSHP recommendations in 2000 (left column) and how they have been updated (right column) is provided in Table 1.1. There are several hypertensive disorders of pregnancy and it is also relevant to take into consideration the presence of any pre-pregnancy hypertension or proteinuria. Specific classifications of these disorders are given in Table 1.1.

Chronic hypertension is classified as hypertension with onset before pregnancy. Pre-eclampsia superimposed on chronic hypertension is defined as the appearance of de novo proteinuria starting after 20 weeks gestation in pregnant women with a prior diagnosis of

chronic hypertension. The diagnosis of white coat hypertension is confirmed by normal BP on 24hr ambulatory BP monitoring (ABPM) in the first half of pregnancy. If white coat hypertension is confirmed, up to 50% of these women can develop gestational hypertension or pre-eclampsia (15).

Gestational hypertension is defined as *de novo* arterial hypertension (systolic BP ≥ 140 mmHg and / or diastolic BP ≥ 90 mmHg on 2 occasions > 6 hours apart) occurring after gestational week 20, which returns to normal post-partum. Pre-eclampsia is often defined as gestational hypertension with proteinuria. Proteinuria is defined as in Table 1.1. Definitions for pre-eclampsia are continuing to change, and for the purposes of this thesis the definition of pre-eclampsia included proteinuria.

Pre-eclampsia can be further defined depending on time of onset or severity of symptoms. The term “early” onset is used to describe pre-eclampsia which is diagnosed before 34 weeks’ gestation. Classification of pre-eclampsia by level of severity is also possible however there are several factors which might contribute to severity and lack of consistency across studies. Examples of severity indicators include small for gestational age babies, or preterm birth at <37weeks gestation. When cohorts are separated based on level of severity of pre-eclampsia, there should be a clear description of this definition on an individual study basis. In an attempt to define severe pre-eclampsia better, the ISSHP circulated a questionnaire to the International Committee of the ISSHP and it was generally agreed that a pre-eclampsia should be defined as “severe” when blood pressure was >160 mmHg systolic, or 110 mmHg diastolic or if haemolysis, elevated liver enzymes and thrombocytopenia (HELLP) syndrome was present (18). Early-onset pre-eclampsia was generally agreed to be diagnosis at less than 34 weeks gestation.

Table 1.1 ISSHP Classification of the Hypertensive Disorders of Pregnancy*

RECOMMENDATIONS 2000	**RECOMMENDATIONS 2014 update**
Classification: **1)** Pre-eclampsia - eclampsia **2)** Gestational hypertension **3)** Chronic hypertension (essential and secondary) **4)** Pre-eclampsia superimposed on chronic hypertension	Classification: 1) Chronic hypertension 2) Gestational hypertension 3) Pre-eclampsia – de novo or superimposed on chronic hypertension 4) White coat hypertension
Pre-eclampsia definition: *De novo* hypertension after gestational week 20, and new onset of one or more of the following: • Proteinuria (as ≥300 mg/day or a spot urine protein/creatinine ratio ≥ 30mg/mmol) • Renal insufficiency (creatinine ≥90umol/L or oliguria) • Liver involvement (↑ transaminases and/or severe right upper quadrant or epigastric pain) • Neurological complications: eclampsia, hyperreflexia with clonus, severe headaches with hyperreflexia, persistent visual disturbances (scotomata) • Haematological disturbances: thrombocytopenia, DIC[#], haemolysis • Foetal growth restriction Normalisation of blood pressure within 3 months postpartum required for definition. Research definition for pre-eclampsia: *De novo* hypertension after 20 weeks gestation, returning to normal postpartum AND properly documented proteinuria	Pre-eclampsia definition: Hypertension developing after 20 wks gestation and the coexistence of one or more of the following new onset conditions (15): **1)** Proteinuria (spot urine protein/creatinine ≥ 30mg/mmol or ≥ 300mg/day or at least 1g/L [‘2+’] on dipstick testing) **2)** Other maternal organ dysfunction: • Renal insufficiency (creatinine ≥90umol/L) • Liver involvement (↑ transaminases and/or severe right upper quadrant or epigastric pain) • Neurological complications (e.g. eclampsia, altered mental status, blindness, stroke, hyperreflexia when accompanied by clonus, severe headaches when accompanied by hyperreflexia, persistent visual scotomata) • Haematological complications (thrombocytopenia, DIC[#], haemolysis) **3)** Uteroplacental dysfunction • Fetal growth restriction

*** Definition of hypertension in pregnancy:**

A systolic blood pressure (BP) ≥ 140mmHg and/or a diastolic BP ≥ 90mmHg on 2 separate occasions. If BP is severely elevated at >160/>110 mmHg, BP should be measured again every 15 minutes, then at 30 minute intervals during initial assessment (18) according to ISSHP recommendations for severe and early-onset pre-eclampsia.

[#]DIC = disseminated intravascular coagulation

1.2 **Pre-eclampsia**

1.2.1 **Pathophysiology of pre-eclampsia**

While the underlying cause of pre-eclampsia is complex and remains poorly understood, most schools of thought support the "two phase" hypothesis which suggests that there is a first phase of the disease, the "placental" phase (with abnormal placentation), followed by a second phase, the "maternal" response (with systemic vascular inflammation). Pre-eclampsia occurs only when a placenta is present, even if there is no fetus (e.g. hydatidiform mole) and usually resolves upon delivery of the placenta (19).

1.2.1.1 ***Placental phase***

As part of the normal process of placentation, cytotrophoblasts cells invade the uterine wall. Cytotrophoblasts are then found in the maternal decidual arteries in the smooth muscle and endothelial layers. Maternal vessels are remodelled from low-flow, high resistance vessels into high-flow, low resistance vessels which supply the placenta and fetus with oxygen and nutrients. A study by Zhou et al found that in order to facilitate this process the phenotype of the cytotrophoblasts changes, and that they express adhesion molecules which are usually found on the surface of endothelial cells (20;21).

An important factor in the pathogenesis of pre-eclampsia is thought to be an impairment of the normal remodelling process of the spiral arteries (22). Cytotrophoblasts do not fully invade and remain present only in superficial layers of the decidua (21). Thus there is failure of the remodelling process of the spiral arteries and this results in high resistance, narrower vessels. The cytotrophoblasts responsible for more shallow invasion in pre-eclampsia have been found by Zhou et al not to exhibit the endothelial adhesion phenotype (23).

When remodelling has failed there is poor quality uteroplacental perfusion caused by higher pressure and lower flow. This leads to an increase in the oxidative stress in the placenta from suboptimal utero-placental flow (24). Over time chorionic villi are injured in a form of ischaemia-reperfusion injury (25). Various factors are released which lead to the maternal syndrome some months later. These include syncytiotrophoblastic membrane

microparticles, factors involved in angiogenesis such as sFlt-1 and soluble endoglin and other factors which lead ultimately to endothelial dysfunction and the features of pre-eclampsia (Figure 1.1).

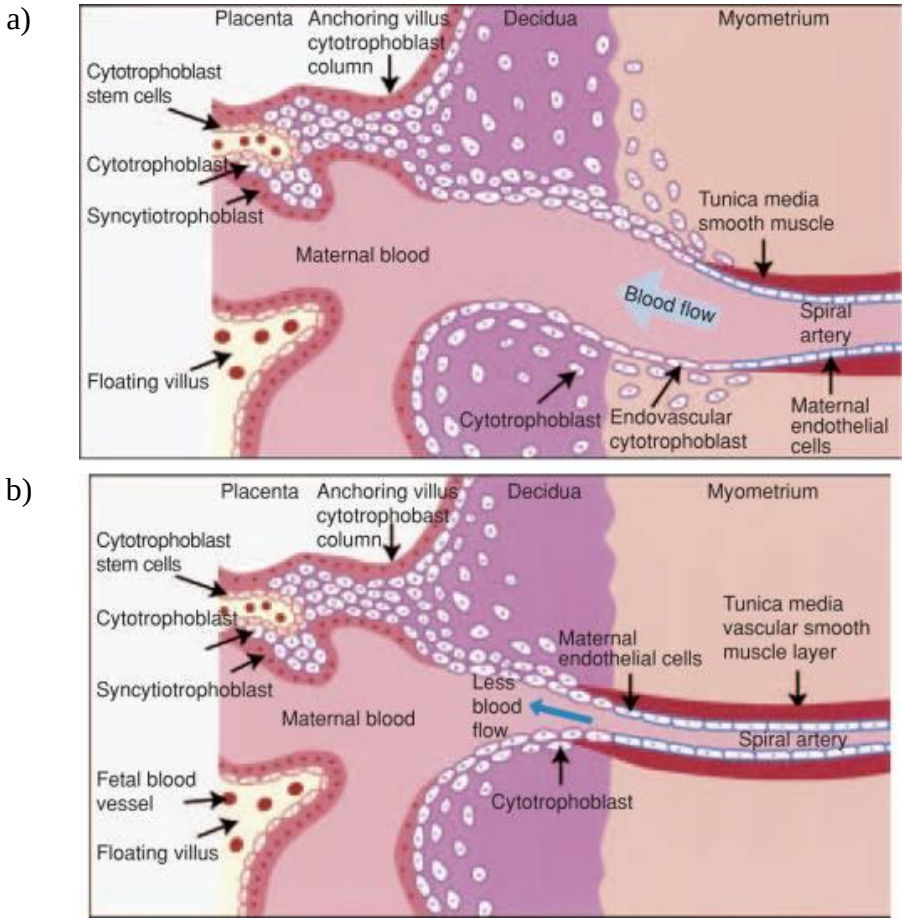


Figure 1.1. Abnormal Placentation in Preeclampsia

1a) In normal pregnancy, cytotrophoblasts differentiate from an epithelial phenotype to an endothelial phenotype. 1b) In pre-eclampsia cytotrophoblasts do not have the invasive endothelial phenotype. This results in shallow spiral artery invasion. Figure from Karumanchi et al with permission (26)

1.2.1.2 *Maternal phase*

Maternal endothelial damage and systemic inflammation cause the features of pre-eclampsia such as hypertension, proteinuria and oedema, seen in the second phase (25) (Figure 1.2). There is leucocyte and complement activation, an acute phase response, coagulation function is disturbed and there is also evidence of hyperlipidaemia and insulin resistance (27).

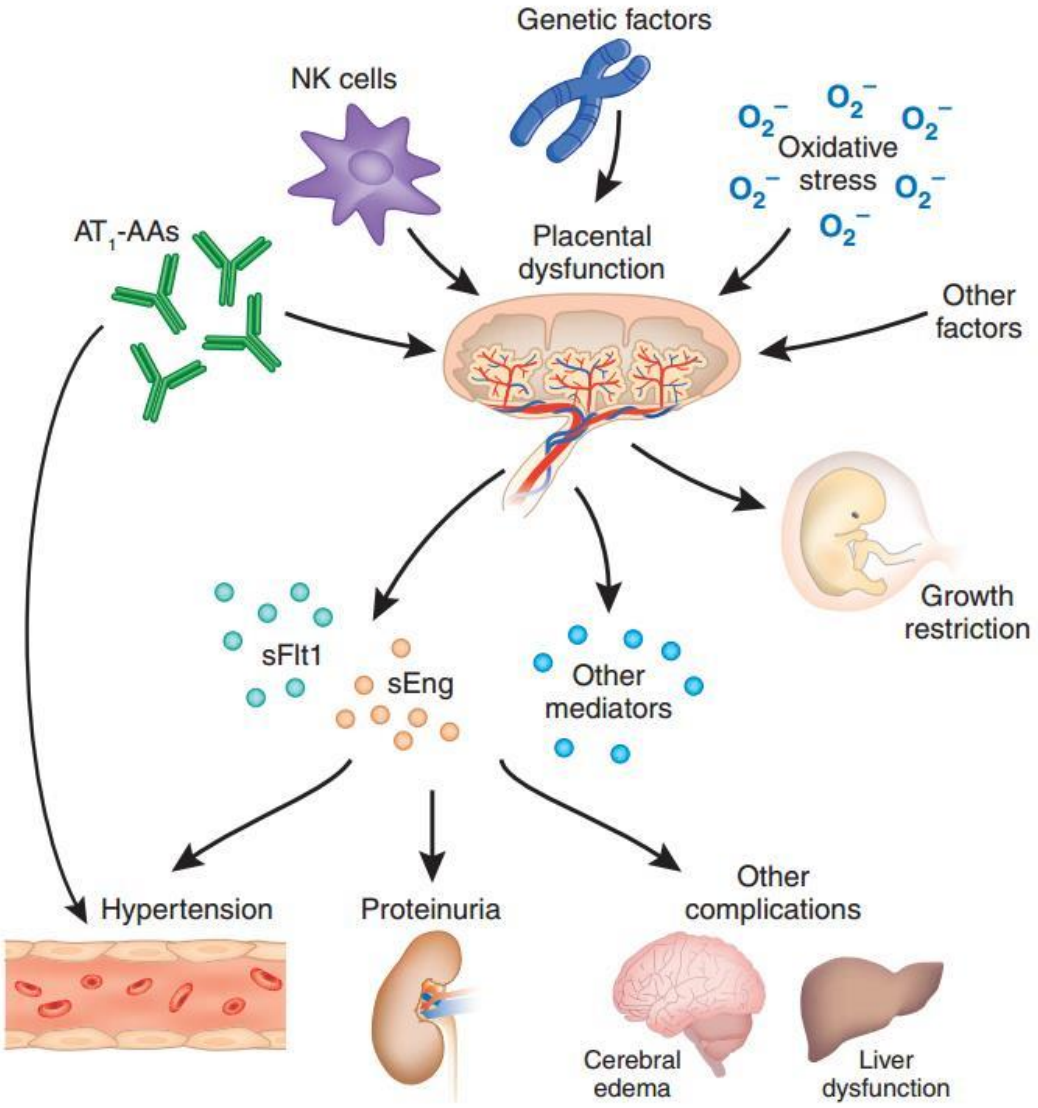


Figure 1.2. Maternal response and the evolution of pre-eclampsia. From Parikh et al with permission (28)

The shape of the placenta in pre-eclampsia may differ from that of normal pregnancy (29). A Finnish study by Kajantie et al examining a large series of placental measurements from 1934-1944 (30) found that the placenta in pre-eclampsia was more oblong-shaped. A circular shape is more usual in normal pregnancy. One hypothesis for the abnormal placental shape, proposed by Burton et al, suggests that normally as part of the trophoblastic invasion process, invasion is strongest at the centre of the placenta and is reduced moving towards the periphery (31). In peripheral areas where invasion is reduced, villi are damaged, partly as a result of excess oxidative stress and partly as a result of mechanical effects (29;31). A circular placenta, with surrounding chorion laevae is formed from the resulting villus necrosis (29). When there is more shallow invasion in pre-

eclampsia, there is more generalized reduced plugging, and the regression of the placenta is not uniform or circumferential with unusual shapes resulting (29).

1.2.2 **Animal models of pre-eclampsia**

There is no perfect animal model for pre-eclampsia which exactly mimics all the human manifestations of the condition. In order to replicate the human experience of pre-eclampsia as closely as possible, a desirable model would have impaired invasion of the trophoblast in the first trimester, develop pregnancy-specific hypertension and proteinuria, and exhibit an imbalance of angiogenic factors and endothelial dysfunction (32). Nevertheless, several animal models have been developed based on the proposed pathophysiological mechanisms of the disease and cover several possible mechanisms of the condition, from reduced uterine perfusion pressure and uteroplacental ischaemia to antagonism of angiogenesis, vasoconstriction, inflammatory models, metabolic models and transgenic and chronic hypertension models to name a few (33). While such models have enhanced our understanding of the condition and can serve as a useful means of testing potential pharmacological interventions (32), cautious interpretation of the results of such studies would be required, especially considering the limitations of these models.

1.2.3 **Risk factors for pre-eclampsia**

Several risk factors for pre-eclampsia have been identified over the years and remain important. As with many other conditions, some of these risk factors are modifiable, others are not. Some risk factors are common to both pre-eclampsia and cardiovascular disease such as diabetes, pre-existing hypertension and renal disease.

Insulin resistance, obesity and systemic inflammation and maternal age greater than 40yrs are all important risk factors (20;34-36). In a recent meta-analysis of the association between body mass index and pre-eclampsia, excess body mass was found to be significantly associated with an increased risk of pre-eclampsia (37). The risk for late-onset pre-eclampsia and gestational hypertension increases by 4% for every year over the age of 32 years and by 10% for every 1 kg/m^2 above a BMI of 24 kg/m^2 (38).

Regarding the pregnancy itself, risk of pre-eclampsia is increased with multiple gestation, with increased placental mass thought to play some role (20;34). In a systematic review of

risk factors, twin pregnancy was found to approximately triple the risk of pre-eclampsia (34). Limited recent exposure to the paternal antigen is also as predisposing risk factor as indicated by increased risk of pre-eclampsia with change in paternity with the latest pregnancy (20;39), use of barrier contraception (40), and assisted conception (41). There are conflicting reports regarding which components of assisted conception are responsible for the increased risk of pre-eclampsia. Ovulation induction, but not *in vitro* fertilisation was associated with an increase in early pre-eclampsia risk in a study by Poon et al (38). A proposed mechanism for this is that ovulation medications decrease the maternal serum concentration of pregnancy-associated plasma protein-A (PAPP-A), and low serum PAPP-A has been associated with early onset pre-eclampsia (42).

Nulliparity nearly triples the risk of pre-eclampsia (34) and women with a history of pre-eclampsia have an increased risk of developing pre-eclampsia again in a subsequent pregnancy (43;44). The risk of recurrence varies depending on other factors such as maternal pre-existing medical disorders, gestational age at time of pre-eclamptic pregnancy and severity of pre-eclampsia (43;45). Longer inter-pregnancy intervals are associated with older maternal age, reduced fertility and the possibility of a change of partner (43). A meta-analysis by Cormick et al found that, when compared with inter-pregnancy intervals of 2-4 years, risk of recurrent pre-eclampsia was increased with longer inter-pregnancy intervals, but shorter intervals were not associated with an increased risk (43).

Regarding family history of pre-eclampsia, a study by Poon et al revealed that independent prediction of pre-eclampsia applied to a history of pre-eclampsia in the mother but not the sister of affected women (38). This study also revealed a nine-fold increased risk of early pre-eclampsia, but there was not an increased risk for late pre-eclampsia, in women with chronic hypertension (38).

Genetic factors play a role in pre-eclampsia, as discussed later in this chapter, but the most obvious evidence of this is that maternal and paternal family history of the disease have been shown to be predisposing factors (46). Ethnicity may also play a role. Black women have been shown to have an increased risk of early-onset pre-eclampsia, and Black and South Asian women an increased risk for late-onset pre-eclampsia, as reported by Poon et al (38).

Smoking during pregnancy has been shown to be protective against pre-eclampsia on systematic review (47). Similar protective findings relating to smoking have also been found in ulcerative colitis (48;49), Parkinson's disease (50) and endometrial cancer in postmenopausal women (51). A potential explanation for this may be the vasoprotective properties of carbon monoxide (CO). *In vitro* culture experiments have shown that CO and CO-releasing molecules lower levels of sFlt-1 and soluble endoglin (49). In a Swedish study of tobacco use in pregnancy and pre-eclampsia risk (52), the effects of cigarette smoking and Swedish snuff on the risk of pre-eclampsia were compared. Cigarette smoking, but not snuff use during pregnancy, was found to decrease the risk of developing pre-eclampsia and gestational hypertension. Nicotine is the common ingredient in snuff and cigarette smoke, so this study lends weight to the concept that protective effects of cigarette smoking are likely mediated by an ingredient in combustion such as carbon monoxide. Nevertheless, the negative impact of smoking on pregnancy in general must not be underestimated. Its benefits in pre-eclampsia are by far outweighed by the damaging effects of smoking on overall health and perinatal outcomes (53).

1.2.4 **Prediction of pre-eclampsia**

There is ongoing research into the role of biomarkers in the prediction of pre-eclampsia, and individual biomarker advances such as sFlt-1/PlGF ratio are mentioned in the biomarker section 1.7.

Some of the more interesting recent advances in this area include the findings of the "Screening for Pregnancy Endpoints (SCOPE)" study, which combined uterine artery Doppler at 19-21 weeks gestation with clinical risk factors and 11 biomarkers measured at 14-16 weeks gestation to predict pre-eclampsia in low-risk nulliparous women (54). The final model for pre-eclampsia prediction included mean arterial pressure, body mass index at 14-16 weeks gestation, the consumption of ≥ 3 pieces of fruit per day, placental growth factor and mean uterine artery resistance index. In training and validation cohorts the performance of this model reported as area under the receiver operator curve (AUC) and 95% confidence interval was AUC 0.73 (0.70 to 0.77) and 0.68 (0.63 to 0.74) respectively (54). A model for early-onset pre-eclampsia which included mean arterial pressure, any pregnancy loss at <10 weeks, angiogenin/placental growth factor ratio and mean uterine artery resistance index revealed AUC 0.89 (0.78 to 1.0) and 0.78 (0.58 to 0.99) in training and validation cohorts respectively. These models, especially in the prediction of term pre-

eclampsia showed only modest prediction and further validation in larger cohorts is desirable.

A metabolomics study evaluating 14 metabolites sampled at 15 weeks gestation in the prediction of pre-eclampsia has also yielded interesting results (55). The 14 metabolites gave an odds ratio of developing pre-eclampsia of 36 (95% CI: 12 to 108) with AUC 0.94. Validation of these findings in a separate cohort gave an odds ratio of 23 (95% CI: 7 to 73) for the 14 metabolites and AUC 0.92. These findings are, not only the first step in exploring the potential of metabolomics in prediction of pre-eclampsia, but also in the discovery of a potential early predictor of pre-eclampsia, measured at just 15 weeks gestation. Such a discovery could have huge implications for the application of clinical resources in a timely fashion, and subsequent improved maternal and fetal outcomes. Further validation work is awaited.

Following on from these advances in research into the prediction of pre-eclampsia is the "Improved Pregnancy Outcomes via Early Detection (IMPROvED)" study (56) which is a multicentre study assessing a proteomic platform and a metabolomic platform in low risk primiparous women. Blood sampling occurs at 15 and 20 weeks gestation with the option of sampling at 11 and 34 weeks. Sampling across all trimesters will also allow gestational age at time of risk assessment to be evaluated. At time of writing this study is still recruiting and the resulting biobank will provide a useful resource for this and future studies.

In women with a diagnosis of pre-eclampsia, predicting serious complications and maternal mortality would be extremely useful. A study by von Dadelszen et al (57) devised a prediction model for adverse maternal outcomes within 48 hours; the fullPIERS (Pre-eclampsia Integrated, Estimate of RiSk) model. Included were features such as chest pain, dyspnoea, oxygen saturation, creatinine and aspartate transaminase concentrations, platelet count and gestational age. The model performed well (AUC ROC 0.88, 95% CI: 0.84 to 0.92). This model is being further validated and also refined for use in lower income settings where resources may otherwise preclude its use.

In conclusion, further validation of prediction models is required (58), however, avenues of current research are exploring the potential of a predictive test, in a timely manner, which is ideally high throughput and cost-effective.

1.2.5 **Prevention of pre-eclampsia**

There is evidence (59;60) that aspirin is useful in the prevention of pre-eclampsia and it is the drug of choice. The NICE guidelines "Hypertension in pregnancy: diagnosis and management" 2010 (2) recommends the use of 75mg aspirin daily from 12 weeks gestation until birth for women at high risk of pre-eclampsia and for women with more than one moderate risk factor for pre-eclampsia. In 2014 the US Preventive Service Task Force recommended the use of aspirin for pre-eclampsia prevention and a recent study by Tolcher et al, to evaluate the incidence of recurrent pre-eclampsia, revealed a decrease of 30% since the 2014 recommendation (60). In a recent multicentre, randomized, placebo-controlled, double-blind trial to investigate low dose aspirin to prevent preterm pre-eclampsia in women at high risk, Rolnik et al (59) found that there was a lower incidence of preterm pre-eclampsia with aspirin vs placebo.

Low molecular weight heparin (LMWH) is also useful in women at increased risk of pre-eclampsia, however, many studies have been too small to draw reliable conclusions (58;61). A meta-analysis by Rodger et al (62) compared LMWH vs no LMWH for prevention of recurrent placenta-mediated pregnancy complications and 18.7% of women given LMWH had recurrent complications vs 42.9% on no LMWH (relative risk reduction 0.52; 95% CI: 0.32 to 0.86). Further research into LMWH would therefore be useful.

In women with low dietary calcium intake, high dose supplementation has been shown to reduce pre-eclampsia (63;64). Dietary supplementation of vitamins C and E and magnesium have not been proven to reduce the risk of pre-eclampsia (58;65;66). According to a Cochrane review (67), vitamin D supplementation requires further assessment (58) as there were only a limited number of high quality trials. In a systematic review of the literature, supplementation with L-arginine in pregnant women with hypertension or who were at risk of pre-eclampsia found a significant reduction in the risk of pre-eclampsia, however the sample size for the outcome was limited and further research is also required (68).

A meta-analysis of dietary and lifestyle interventions in pregnancy (69) revealed that weight management was associated with an overall reduction in pre-eclampsia of 26% (RR 0.74, 0.60 to 0.92, p=0.006) and dietary interventions led to a 33% reduction in the risk of

pre-eclampsia (RR 0.67, 0.53 to 0.85, P<0.001). However, the studies had limited information on duration of intervention, intensity of intervention and method of providing the service, therefore implementation of these interventions could be difficult to provide and maintain, and there may be issues of patient compliance.

In summary, there is evidence for the use of aspirin to prevent pre-eclampsia, however, all other interventions mentioned require further assessment.

1.2.6 **Management of pre-eclampsia**

Pre-conception counselling in women who have risk factors for developing pre-eclampsia would be the ideal scenario, however global data for 2012 suggest that approximately 40% of all pregnancies worldwide are unplanned (70). The number of women who have chronic hypertension when they become pregnant is also increasing (71;72) as is the frequency of women developing gestational hypertension during pregnancy (73). It is likely that increasing age at first pregnancy and higher BMI has contributed to these trends.

The NICE guidelines for hypertension in pregnancy, published in 2010 and updated in 2011, outline management of pre-eclampsia (2). They are currently under further review as of 2017. Women with mild (BP 140-149/90-99 mmHg), moderate (BP 150-159/100-109 mmHg) severe (BP ≥160/110 mmHg) hypertension with pre-eclampsia should be admitted to hospital. Treatment is suggested for those pre-eclamptic women with moderate and severe hypertension, aiming to keep systolic blood pressure <150mmHg and diastolic blood pressure 80-100 mmHg. Oral labetolol is suggested as first-line treatment, with methyldopa and nifedipine offered as potential alternatives depending on the woman's side-effect profile. Blood pressure should be measured four times a day at least in mild to moderate cases and more frequently in severe cases. The guideline does not suggest repeat quantification of proteinuria. Blood tests for kidney function, electrolytes, full blood count, bilirubin and transaminases are suggested twice a week in mild pre-eclampsia and three times a week in moderate and severe cases (2).

Regarding the timing of delivery, women should be managed conservatively until 34 weeks and senior obstetric staff should document a plan of criteria for elective birth prior to this time. A plan for antenatal fetal monitoring during birth should also be documented. In cases of pre-eclampsia with severe hypertension after 34 weeks gestation, birth is

recommended once a course of corticosteroids is completed (if required) and blood pressure is controlled. Women with mild to moderate hypertension and pre-eclampsia should be offered delivery between 34^{+0} – 36^{+6} weeks as appropriate depending on the condition of mother and fetus and the availability of neonatal intensive care. For women at 37^{+0} weeks gestation or beyond who have mild to moderate hypertension with pre-eclampsia, birth is recommended within 1-2 days. Blood pressure should be followed-up along with monitoring of haematological and biochemical parameters.

For women in a critical care setting with severe pre-eclampsia or severe hypertension, intravenous magnesium sulphate should be given if they have or previously had an eclamptic fit. Anti-hypertensives suggested are labetolol, hydralazine or nifedipine and response to treatment must be monitored with the aim to keep systolic blood pressure <150 mmHg and diastolic blood pressure 80-100 mmHg. If birth is considered to be likely within 1 week, betamethasone is administered to aid maturation of the fetal lung.

Regarding follow-up after delivery, in women with gestational hypertension or pre-eclampsia, they should be informed that they are at increased risk of developing hypertension and its sequelae later in life. This information should also be forwarded to the primary care team (2). The guidelines also state that women with pre-eclampsia should be informed that they have a recurrence risk of pre-eclampsia of 1 in 6 in a future pregnancy, however this risk is increased to 1 in 4 if they had severe pre-eclampsia, eclampsia or HELLP syndrome with birth at <34 weeks gestation. In births at <28weeks, the risk increases again to 55%. Inter-pregnancy interval of <10 years does not confer any additional risk of pre-eclampsia (2).

Topical issues in the management of women with hypertensive disorders of pregnancy and pre-eclampsia pertain to the optimum blood pressure to aim for in these women and the optimum timing of delivery. There has been concern over excessive blood pressure reduction in pregnancy and the concept that it might further reduce utero-placental perfusion causing lower birth weight. (74). A meta-regression analysis by von Dadelszen et al reported that a 10 mm Hg fall in maternal mean arterial pressure was associated with a 176g decrease in birth weight (74).

In order to further investigate the effect of blood pressure control on perinatal and maternal outcomes an international multicentre randomised controlled trial, “the Control of

Hypertension in Pregnancy Study (CHIPS)" was set up (75). There has been concern about the potential of lowering blood pressure to be harmful and the possibility of it impacting not only on fetal growth but also on perinatal morbidity and mortality. The CHIPS trial sought to investigate women with non-proteinuric, non-severe pre-existing hypertension or gestational hypertension, women with live singleton fetus at 14weeks to 33weeks 6 days gestation, and a diastolic blood pressure of 90-105mmHg if on no antihypertensive therapy or 85-105mmHg if they were on antihypertensive therapy. Women were randomly assigned to either less-tight control (target DBP 100 mmHg) or tight control (target DBP 85 mmHg). In both groups just over 50% of the women were taking antihypertensive medication at enrolment. Labetolol was the antihypertensive medication suggested in the study protocol, however only two thirds of women on antihypertensive medication were on this agent. While women in the less-tight control group had a higher rate of severe maternal hypertension, there were no significant differences in risk of adverse perinatal outcomes or overall serious maternal complications. Secondary analysis of the CHIPS data (76) revealed that after adjusting for pre-eclampsia, severe hypertension was a significant risk factor for adverse perinatal and maternal outcomes. The CHIPS trial had a total study population of less than 1000 participants. Further studies would assist in further clarification regarding optimum blood pressure targets and their potential benefits.

Regarding timing of delivery, this has been investigated in the first HYPITAT study (77), which was a multicentre randomised controlled trial investigating induction of labour in women with a singleton pregnancy complicated by gestational hypertension or mild pre-eclampsia at 36-41 weeks gestation. It found that adverse maternal outcomes were reduced with delivery after 37 weeks gestation, and neonatal outcomes were not significantly different. The HYPITAT-II trial investigated delivery in women with hypertensive disorders of pregnancy between 34 and 37 weeks gestation and found that while the small risk of adverse maternal outcomes was reduced, there was a significant risk of neonatal respiratory distress syndrome (78). Risks to fetus must be weighed up in addition to maternal risks in considering delivery at 34-37 weeks gestation.

1.3 **Cardiovascular risk after pre-eclampsia**

Women with a history of hypertensive complications in pregnancy are known to be at an increased risk of vascular and metabolic diseases later on in life (79). One of the first suggestions of a link between pre-eclampsia and future cardiovascular disease was

reported ~55 years ago (80). Since then more and more evidence has accumulated in favour of postpartum cardiovascular risk in women with a history of pre-eclampsia (81-85). In a recent study by Behrens et al (86), in comparison with women with normotensive pregnancies, women who had a hypertensive disorder of pregnancy in their first pregnancy were found to have an increased risk of developing hypertension during the following 10 years of 14-32% depending on age at time of pregnancy. Recurrent pre-eclampsia is associated with greater risk of cardiovascular disease, and women who had pre-eclampsia associated with fetal growth restriction or developed pre-eclampsia before 34 weeks gestation have four to eight times the risk of cardiovascular death in comparison with women who had a normal pregnancy (87;88). The impact of pre-eclampsia on future cardiovascular health has now been acknowledged by the American Heart Association, which recognises pre-eclampsia as a risk factor for cardiovascular disease (89) and stroke (90) in its latest guidelines regarding cardiovascular risk in women.

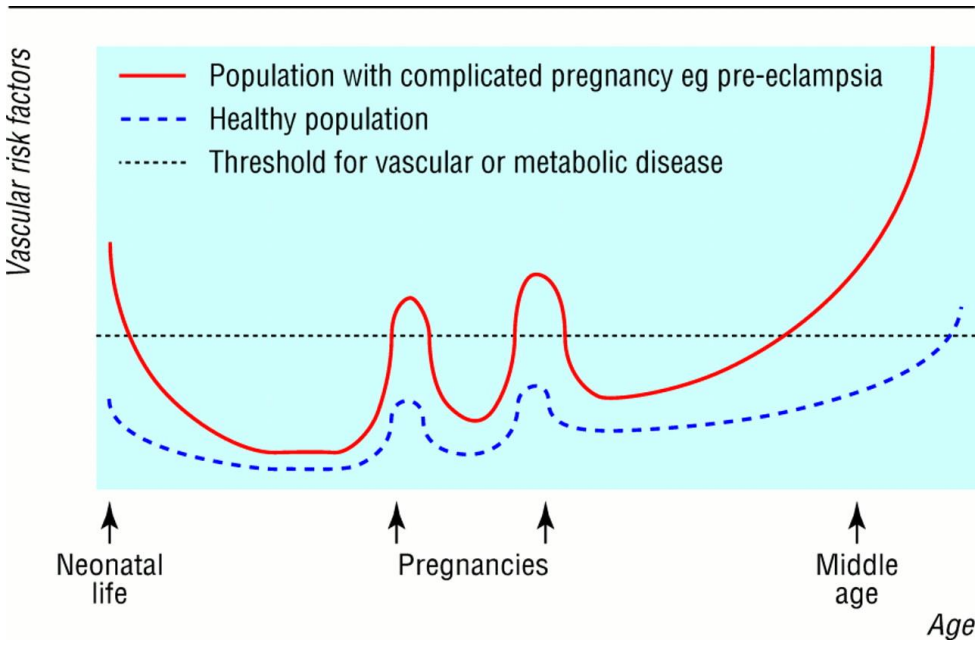


Figure 1.3 Increased vascular risk with complicated pregnancy
Women with a history of complicated pregnancy e.g. pre-eclampsia (red line) have greater risk of developing vascular and metabolic disease than women with healthy pregnancies (blue line). Figure from Sattar et al with permission (79).

Shared risk factors for pre-eclampsia and cardiovascular disease include obesity, insulin resistance and lipid abnormalities. Endothelial dysfunction is a key component of both. In normal pregnancy there is upregulation of inflammatory markers, an increase in coagulation factors, a degree of insulin resistance and hyperlipidaemia (91). In pre-

eclampsia these normal adaptive mechanisms are enhanced (92). Oxidative stress also contributes to endothelial dysfunction in atherosclerosis and in pre-eclampsia.

1.3.1 **Epidemiological studies**

Many epidemiological studies in various populations comprising various ethnicities have reported the associations between pre-eclampsia and cardiovascular disease (93), and these studies are getting larger and larger. A large study of 1.03 million women in Ontario Canada, the CHAMPS study, found that women with pre-eclampsia, who did not have any evidence of cardiovascular disease at the beginning of pregnancy were found to have a hazard ratio of 2.1 of developing future cardiovascular disease in comparison with women who did not have a history of pre-eclampsia (83). Another large study in Taiwan demonstrated that women with a history of pre-eclampsia/eclampsia had an increased risk of major cardiac events up to 3 years post-partum (94).

Various studies in other parts of the world have drawn similar conclusions; USA (95;96), Nordic countries (81;97;98), UK (99) and Scotland (85;100). One of the Scottish studies by Smith et al (85) used routine discharge data to link all singleton first births from 1981-1985 with the mothers' subsequent admissions and deaths for a 15-19yr follow-up period. Delivering a baby in the lowest birthweight quintile for gestational age gave an adjusted hazard ratio 1.9 [95% CI 1.5-2.4] of developing maternal ischaemic heart disease or death. Preterm delivery, and pre-eclampsia gave adjusted hazard ratios [95% CI] of 1.8 [1.3-2.5] and 2.0 [1.5-2.5] respectively. Women with all three characteristics had a risk of IHD admission or death seven times greater than the reference category. Bellamy et al (6) performed a systematic review of the literature and meta-analysis and reported on the relationship between pre-eclampsia, cardiovascular diseases and cancer. Women with a history of pre-eclampsia had relative risk of 3.70 (2.70 to 5.05) of developing hypertension, 2.16 (1.86 to 2.52) of developing ischaemic heart disease and 1.81 (1.45 to 2.27) of developing stroke in comparison with women who had normal pregnancies. They were followed up for 14yrs, 11yrs and 10yrs respectively and there were no differences in rates of cancer in general or breast cancer between groups. Another systematic review and meta-analysis, by McDonald et al (101), also reported that in women with a history of pre-eclampsia there was approximately a 2-fold increase in risk of stroke, ischaemic heart disease and cardiovascular death in women with a previous history of pre-eclampsia. In a systematic review and meta-analysis of cardiovascular disease in women with pre-

eclampsia by Brown et al, a significant increase in odds of fatal or diagnosed cardiovascular disease, hypertension and cerebrovascular disease was found (102). The odds of having such an event was also estimated as being double that of women without pre-eclampsia.

Regarding hypertensive disorders of pregnancy without proteinuria, a recent large Finnish study (82) has suggested that in patients with any hypertensive disorder of pregnancy, there was an increase in cardiovascular disease risk including myocardial infarction (MI), MI death, ischaemic heart disease, stroke and heart failure.

1.3.2 **Causes of cardiovascular risk after pre-eclampsia**

There is debate as to whether pre-eclampsia itself causes cardiovascular disease or whether pregnancy reveals a woman's underlying and pre-existing risk of cardiovascular disease (Figure 1.3).

New evidence for the direct effect of pre-eclampsia itself on cardiovascular risk has recently emerged from animal studies. In a study by Pruthi et al (88) after exposure to a mouse model of sFlt-1 induced pre-eclampsia, a mouse carotid injury model was used, to test the hypothesis that there is an enhanced vascular response to future vessel injury with prior exposure to pre-eclampsia. Mice exposed to pre-eclampsia did exhibit an enhanced responsiveness to the carotid injury model in comparison with mice not exposed to pre-eclampsia (88). These findings support the concept of pre-eclampsia itself changing the response to future vascular insult.

However, evidence in support of the hypothesis that there may be pre-pregnancy risk factors common to both pre-eclampsia and cardiovascular diseases has been provided by the Norwegian population-based Nord-Trondelag Health Study (HUNT) (103) where longitudinal data from two different time-points were linked with Medical Birth Registry of Norway data. Data available from HUNT 1 (1984-1986), and HUNT 2 (1995-1997) included measurement of height, weight, blood pressure and lifestyle factors such as socioeconomic status and smoking history. Non-fasting serum lipid measurements had also been made at the time of HUNT 2. Women with a history of pre-eclampsia or gestational hypertension had higher systolic and diastolic blood pressure measurements, higher BMI and unfavourable lipid profiles. On adjustment for pre-pregnancy measurements, there was

attenuation of the difference in BMI, blood pressure, triglycerides and HDL cholesterol. The results suggest there were risk factors for later cardiovascular disease present before the pregnancies themselves. They do not, however, completely exclude the possible role of a hypertensive or pre-eclamptic pregnancy on later cardiovascular disease. Further longitudinal studies may help to further clarify the role of pre-pregnancy status on risk of cardiovascular disease after hypertensive disorders of pregnancy.

There are a vast spectrum of study types which tackle various aspects of the pathophysiology and management of disease in pre-eclampsia, cardiovascular disease itself and cardiovascular disease in women with a more remote history of pre-eclampsia. Various investigative approaches are common to all three of these study areas (see Fig 1.4). In order to clarify outstanding questions in the pathophysiology of pre-eclampsia and underlying mechanisms of future cardiovascular disease risk after pre-eclampsia further research is required.

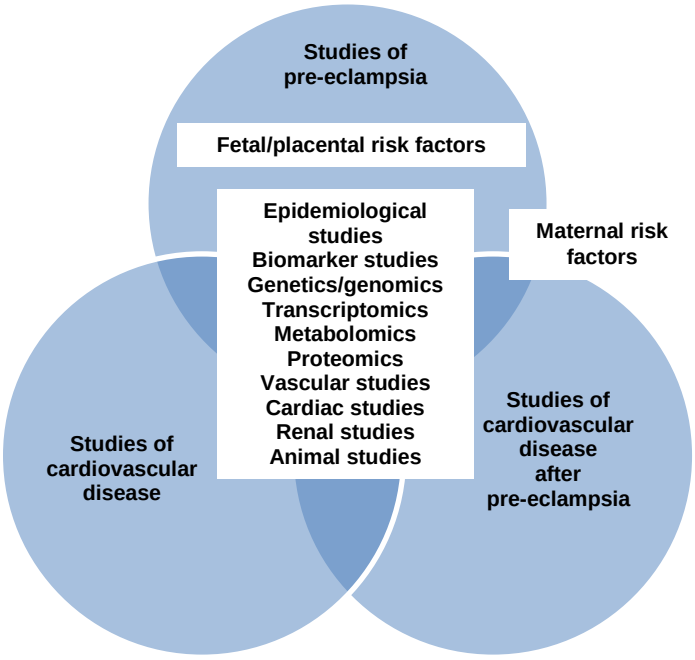


Figure 1.4 Types of studies investigating pre-eclampsia, cardiovascular disease and the relationship between them

Rather than separating the link between pre-eclampsia and cardiovascular disease as being from the metabolic disturbance and endothelial damage initiated by the onset of pre-eclampsia itself or by pre-existing and pre-pregnancy susceptibility to pre-eclampsia and later cardiovascular disease, it is more likely that a combination of these two concepts underlies the overall cardiovascular disease susceptibility.

1.3.3 **Cardiovascular risk in offspring of women with pre-eclampsia**

While pregnancy is now being viewed as a critical period of time in which to assess a woman's future cardiovascular risk, a window to her cardiovascular future, it is also important to offspring cardiovascular risk (104).

A systematic review by Davis et al (105) of traditional cardiovascular risk factors in offspring of pre-eclamptic pregnancies vs controls revealed that during childhood and young adulthood, systolic blood pressure was 2.39 mmHg (95% CI: 1.75-3.05, P<0.0001) higher and diastolic blood pressure 1.35 mmHg (95% CI: 0.90-1.80, P<0.00001) higher. BMI was also higher in children exposed to a pre-eclamptic in-utero environment compared with controls (0.62kg/m^2, P<0.00001).

A UK-based study of children aged 9-12 years (106) found evidence of 2.04 mmHg (95% CI:1.33,2.76) higher systolic blood pressure and 1.10 mmHg (95% CI: 0.47, 1.73) diastolic blood pressure in children who were offspring of pre-eclamptic pregnancies, after adjusting for maternal and offspring confounders such as BMI.

One explanation for these findings would be Barker's hypothesis that an increased risk of hypertension and cardiovascular disease may result from exposure of the developing fetus to an adverse in-utero environment during a critical period of development (107).

However, contradictory to this hypothesis is a recent study by Alsnes et al (108) which found that while offspring born to mothers with hypertensive disorders of pregnancy exhibited greater cardiovascular risk in adulthood than offspring of normotensive pregnancies, the cardiovascular risk to siblings born to the same mother but following a normotensive pregnancy were the same. The findings of this study were in favour of a genetic or lifestyle influence being stronger in determining offspring cardiovascular risk than maternal blood pressure in pregnancy. This has implications for future risk assessment

for cardiovascular disease after hypertensive pregnancy. It promotes the concept that offspring should also be followed up regarding their own cardiovascular risk, regardless of whether their mother experienced hypertension specifically in her pregnancy with them, but rather if she experienced hypertension in any pregnancy.

1.4 Vascular function in pre-eclampsia

1.4.1 Endothelial function

Whilst there are several non-invasive methods for measuring endothelial function in research, flow-mediated dilatation (FMD) has been one of the most widely used, especially in the evaluation of women with a history of pre-eclampsia. However, endothelial function can also be assessed by means of peripheral arterial tonometry (PAT) and laser doppler imaging (and iontophoresis) which have also been used to investigate women with a history of pre-eclampsia, and both of which are less operator dependent than FMD. All three of these modalities will be addressed in this section.

1.4.1.1 *Flow-mediated dilatation*

In flow-mediated dilatation the change in artery diameter in response to reactive hyperaemia is measured (109). Specialised ion channels in the endothelial cell membrane open in response to shear stress (110) and calcium entry is increased. The calcium activates the enzyme endothelial nitric oxide synthase (eNOS), and the generation of nitric oxide which subsequently occurs results in flow-mediated dilatation. FMD was first proposed as a tool for assessment of endothelial function in 1992 and since then there have been further technological and methodological advances in protocols (110-114). The limitations of FMD are widely acknowledged (109;115) and while as a tool it has great merit in the research setting, its use in the clinical arena is precluded at the present time. While FMD is known to be highly operator dependent, several studies have assessed reproducibility with reassuring results and suggest that its use in clinical studies is acceptable (116-119). While in most studies FMD is performed at the brachial artery, it can also be performed at the radial or femoral arteries (109). During a normal FMD protocol, duplex ultrasound is used to measure baseline artery diameter and velocity of blood flow.

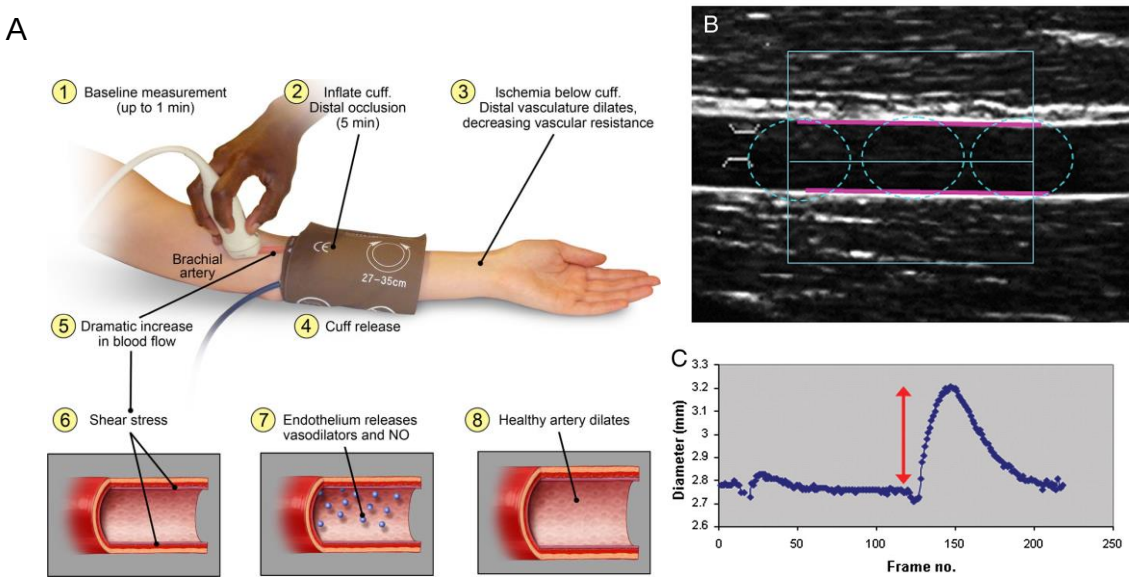


Figure 1.5 Flow-mediated dilatation. A) The FMD protocol typically involves at least 1 minute of baseline measurement, 5 minutes of distal cuff occlusion, and up to 5 minutes of data collection following release of the cuff. From Weissgerber TL et al (112), B) Analysis software showing region of interest box. From Charakida M et al (113), C) Edge detection software generated output. From Charakida M et al with permission (113).

The occlusion cuff is inflated to suprasystolic pressure for 5 minutes. When it is released, the reduction in downstream resistance (caused by dilatation of the distal vessels in response to the ischaemia in tissue distal to the cuff) results in increased blood flow to the arm. The resulting increase in shear stress causes the endothelium to release vasodilators which in turn normally cause dilatation in healthy vessels. Dilatation is reduced or absent in patients with vascular dysfunction (112). FMD is calculated as the percentage change in artery diameter. Maximum dilatation tends to occur at ~45-60 seconds post-release in young healthy individuals (109;112), however there can be marked variability between individuals and peak diameter can occur later in individuals with disease or older individuals (120). The procedure is outlined in Figure 1.5.

Previous studies, including a meta-analysis, have shown that brachial FMD can predict incident cardiovascular events in middle-age to older adults (121;122) and in patients with obstructive coronary artery disease, it has been shown to be predictive of future adverse events (123).

Endothelium-dependent FMD can be used to assess vascular function in women with pre-eclampsia (109;112). In most studies in women with pre-eclampsia, FMD is reduced in women with pre-eclampsia in comparison with women who were normotensive during pregnancy (112;124-131). There were some studies which did not report a difference between women with pre-eclampsia and normotensive women (112;132-134). However, in a recent systematic review and meta-analysis of FMD before, during and for 3 years after pre-eclampsia, 37 studies were eligible for meta-analysis. The findings indicated that women with a history of pre-eclampsia had lower FMD before the development of pre-eclampsia, at the time of pre-eclampsia and for 3 years post-partum, in comparison with women who did not have pre-eclampsia (135). On further investigation of FMD after pre-eclamptic pregnancy, some studies suggest that in the initial 4-6 weeks post-partum period, FMD is actually increased in women with pre-eclampsia (128;132) suggesting an initial reversal of the endothelial dysfunction. Studies investigating the post-partum period up to 3 years tend to suggest FMD will remain lower in women with a history of pre-eclampsia (127;134;136-140) in comparison with normotensive women. Some studies suggest the timing and severity of pre-eclampsia may have an impact on FMD with early-onset or pre-term pre-eclampsia having lower FMD measurements (112;127;141). FMD has also been found to be lower in women with recurrent pre-eclampsia in comparison with women who had one pre-eclamptic pregnancy (136).

By 10 or more years post-partum, two studies have shown that vascular dysfunction no longer persists and there is normalisation of FMD. For example, a study by Ostlund et al reports that women who had pre-eclampsia and were evaluated at 1 year post-partum were found at that time to have reduced FMD, but when they were evaluated again at 11 years post-partum they were found to have FMD values similar to women with normotensive pregnancies (142). However, the women with pre-eclampsia, at this later time point, had evidence of impaired glucose tolerance and higher blood pressures than their normotensive counterparts (142). The other study, assessing endothelial function in women 10 years after a pre-eclamptic first pregnancy (89 women) vs first pregnancies without pre-eclampsia (69 women) also concluded that pre-eclampsia was not associated with impaired FMD 10 years after pregnancy in previously healthy women, however in this study pre-eclampsia was associated with changes in circulating markers representative of possible early endothelial dysfunction (143).

1.4.1.2 *Peripheral arterial tonometry (PAT)*

This non-invasive method uses fingertip pulse amplitude tonometry to evaluate endothelial dysfunction by measuring the changes in digital pulse volume throughout reactive hyperaemia (144;145). It is measured with an endothelial Peripheral Arterial Tonometry (EndoPAT) device. First a blood pressure cuff is applied to the dominant arm. Pneumatic finger probes are then applied to the index finger of each hand and a recording of the PAT signal is commenced for 5-10 minutes baseline recording (depending on study protocol) (144;145). Occlusion of the brachial artery is achieved by inflation of the blood pressure cuff to suprasystolic pressures for 5 minutes. The other arm acts as a control. On release of the blood pressure cuff, PAT recording continues for another 5 minutes. The reactive hyperaemia index (RHI) is calculated by dividing the average amplitude of the PAT signal over a specified time-period after cuff deflation, with the average amplitude over a specified time before the cuff inflation. The control arm is used to correct for other factors such as room temperature, and the RHI is calculated by EndoPAT software. The EndoPAT device is also capable of recording augmentation index, a measure of arterial stiffness.

A previous study has shown that an impaired hyperaemic response is associated with endothelial dysfunction of the coronary arteries (144;146), and RHI has also been associated with several traditional and metabolic risk factors in the Framingham Heart Study (147-149).

In a study of endothelial function in pregnancy, using EndoPAT (145), pregnant women who had risk factors for developing pre-eclampsia were examined at gestational weeks 16 and 28 and again between 6-9 months postnatally. In women who had developed pre-eclampsia or pregnancy-induced hypertension, the postnatal baseline pulse amplitude was lower than in normotensive controls. However, during pregnancy there had been no difference between cases and controls at either 16 weeks or 28 weeks, therefore EndoPAT had not been able to predict pre-eclampsia. Both groups had shown a higher baseline pulse amplitude at week 28, but it was not until the postnatal period that any differences in pulse amplitude emerged between groups. RHI was not significantly different between groups at any time point.

Two more recent studies have assessed women with pre-eclampsia during pregnancy (150) and between 6 months and 4 years after delivery (151). Both showed evidence of

endothelial dysfunction with statistically significantly reduced RHI and both showed an increase in arterial stiffness as measured by augmentation index with the EndoPAT device. These findings are consistent with a previous study by Yinon et al (152) which demonstrated evidence of endothelial dysfunction in pregnant women with pre-eclampsia in comparison with normotensive controls.

1.4.1.3 ***Laser Doppler imaging and iontophoresis***

In laser Doppler imaging, a monochromatic laser is scanned across the skin surface and light is backscattered from moving erythrocytes. The light undergoes a shift in frequency which is proportional to the velocity of the erythrocytes (153;154). The resulting image is colour-coded and represents the skin blood flow over the area which is being scanned. Each image is processed using software which calculates the laser Doppler flux measurement given in flow units. Drugs are administered via iontophoresis which follows the principle that charged molecules of a drug solution will migrate across the skin under the influence of a current which is applied (154;155). Acetylcholine which vasodilates in an endothelium-dependent manner and sodium nitroprusside which vasodilates in an endothelium-independent manner are the more commonly used drugs in this procedure.

In a recent study microvascular function was evaluated in women in the third trimester of uncomplicated pregnancies, and was re-evaluated at 6 weeks and 6 months postpartum (156). This study found that acetylcholine-mediated vasodilation was increased in normal pregnancy and returned to pre-pregnant levels by 6 months postpartum, however, in women with pre-eclampsia levels did not decrease in the postpartum period but remained high. The findings of increased microvascular responses in women with pre-eclampsia are mostly consistent with other studies (153;155-157), the only difference being that Khan et al (153), who examined women by laser Doppler imaging at 22 weeks, 26 weeks, 34 weeks gestation and 6 weeks postpartum did not find differences between pre-eclamptic and normotensive pregnancies in the postpartum period. Microvascular responses during pregnancy had been higher in pre-eclamptic than normotensive women.

Overall, these results contradict the findings of larger vessels such as the brachial artery, where during the same time periods, FMD shows a decreased response in relation to pre-eclampsia. One hypothesis for this disparity is that endothelial dysfunction is expressed differently between macro- and microvessels (153) during pregnancy and the post-partum

period. In a study of women with a much more remote history of pre-eclampsia, 15-25 years after pregnancy (158) there was a decrease in acetylcholine response which it concluded was indicative of impaired endothelial function. The interpretation of these findings as impaired endothelial function in this context is similar to other studies which found reduced response to acetylcholine and sodium nitroprusside in coronary heart disease risk (159) and type 1 diabetes (160).

1.4.2 Vascular stiffness

1.4.2.1 *Pulse wave velocity*

Pulse wave velocity (PWV) is related to the intrinsic elasticity of the arterial wall and its anatomic dimensions (161;162). The speed of the reflected arterial wave increases as arteriolar constriction occurs or arterial stiffness increases (162). PWV increases from 4-5 m/s in the ascending aorta to 8-9 m/s in the iliac and femoral arteries (162). Disease and aging can reduce the elastic component of the arterial wall, therefore arterial stiffness tends to increase with age (163). Endothelial function can affect wall stiffness, by altering the integrity of the extracellular matrix or smooth muscle tone (163). Pulse wave velocity itself is calculated from the distance between two recording sites, and the time delay between corresponding points of the pressure wave (164). The main benefits of this method of assessment are that it is reproducible, non-invasive and simple to perform.

In 2015 the American Heart Association issued recommendations for improving research on arterial stiffness (163). Recommendations for devices used to measure PWV are that arterial stiffness should be determined noninvasively by measurement of carotid-femoral PWV (cfPWV) (163;165;166). The measurement of PWV in other areas such as carotid-radial was not recommended as there was no evidence that it predicted outcomes (163;167). Carotid-femoral PWV was also thought to inform risk stratification above that provided by traditional cardiac risk factors in people who are at intermediate risk of cardiovascular disease (163;165).

In the 2007 European Society of Hypertension/European Society of Cardiology (ESH/ESC) guidelines for the management of hypertension (168) a fixed cut-off for cfPWV of 12m/s to indicate subclinical organ damage was suggested. However, a more recent consensus statement proposed the use of 10m/s as the cut-off, as this took into

consideration a new distance calculation method (169). The 2015 AHA recommendations warn of the hazards of using a fixed threshold e.g. the effects of age on PWV (163).

In a very small study of pregnant women, comprising 10 normotensive, 8 hypertensive and 8 pre-eclamptic pregnancies, cfPWV was higher in the 16 hypertensive pregnant women than the 10 normotensive (p-value <0.001) (170). In a systematic review and meta-analysis published in 2012, the association between pre-eclampsia and arterial stiffness was examined and 23 studies were included (171). Women with pre-eclamptic pregnancies had a significant increase in cfPWV and augmentation index (discussed below in the section on pulse wave analysis) in comparison with women who had normotensive pregnancies (171). This increase in arterial stiffness was noted prior to, during and in the months following pre-eclamptic pregnancy. The difference between pre-eclamptic cases and normotensive controls was larger in early-onset and severe pre-eclampsia.

This study concluded that arterial stiffness measurements may be useful in predicting the onset of pre-eclampsia, and arterial stiffness may have a role in the increased risk of cardiovascular disease later in life (171).

1.4.2.2 ***Pulse wave analysis***

Pulse wave analysis (PWA) recordings are generated from pulse pressure waveforms obtained with applanation tonometry. Applanation tonometry is simple to perform, non-invasive, reproducible and is validated (172). It is a technique which allows assessment of the various components of the pulse wave. The central aortic pressure wave comprises a forward-travelling wave, which is initiated by left ventricular ejection, and a later wave which is reflected from the periphery (173;174) (Figure 1.6). Different arterial sites give rise to different pulse waveforms. Also, as arterial stiffness increases, the velocity of the forward and reflected waves increases, and the reflected wave arrives earlier and augments the pressure in late systole. Augmentation pressure is defined as the difference between the second and first systolic peak, and augmentation index (AIx) is the augmentation pressure divided by the pulse pressure expressed as a percentage (174). Central aortic waveforms are derived from the radial artery waveform using a generalised transfer function. Heart rate is known to influence AIx (175), therefore it is normalised to a heart rate of 75 beats per minute (AIx@75).

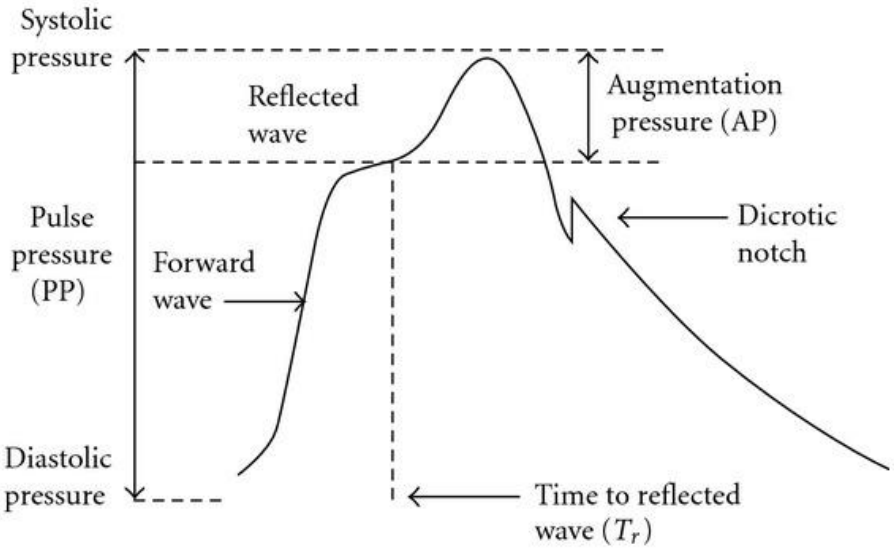


Figure 1.6 The pulse wave and derived functions
The augmentation pressure (AP) is the pressure difference between the maximal systolic peak and the inflection point. Augmentation index is calculated as AP divided by pulse pressure (PP). From Stoner et al with permission (176).

A study by Weber et al reported that non-invasively measured AIx and augmentation pressure were independent risk markers for coronary artery disease (174).

In women with a history of pre-eclampsia, AIx is increased in comparison with women who have other hypertensive disorders of pregnancy without proteinuria (177). Another study examining women a few years postnatally found no difference in augmentation index between women who had pre-eclampsia and those who had normal pregnancies (178). However, a recent meta-analysis of markers of vascular dysfunction after hypertensive disorders of pregnancy (179) found that women with a history of hypertensive disorders of pregnancy had a higher AIx than women with normotensive pregnancies.

1.4.3 **Carotid ultrasonography**

Carotid ultrasound is a non-invasive safe method of measuring carotid intima-media thickness (IMT) and detecting carotid plaques. It has been validated for use in assessing the presence of pre-clinical atherosclerosis and the procedure is reproducible and simple to perform with several protocol guidelines in existence (180-182). Carotid intima-media thickness is measured as the distance between the luminal-intima and media-adventitial interfaces according to the original paper by Pignoli et al (183).

Over the past decade guidelines have varied in their recommendations. In 2010, the American Heart Association/American College of Cardiology (AHA/ACC) guideline recommended that in intermediate risk asymptomatic adults, carotid IMT measurement could be used for assessment of cardiovascular disease risk (115). This was a class IIa recommendation. In 2013 however, the AHA/ACC guidelines recommended against the use of carotid IMT in predicting individual risk in clinical practice (184). The European Society of Hypertension/European Society of Cardiology recommend carotid ultrasound for the detection of atherosclerosis or vascular hypertrophy as a class IIa recommendation with level of evidence B (185), and a Mannheim Consensus update regarding carotid intima-media thickness and plaques recommended measurement of carotid IMT and plaque presence for detection of cardiovascular risk in individuals who were asymptomatic but at intermediate risk or if risk factors were present (182).

In the past, studies have shown that there is not any significant prognostic value found in combining carotid IMT with classic risk factor scores, such as the Framingham Risk Score (186-189). Despite this, there have been many studies which have shown an association between carotid IMT and the risk of cardiovascular events in the future (190-193). To further complicate matters, the findings of two meta-analyses on the subject, by Lorenz et al and Den Ruijter et al were inconsistent (194;195). Lorenz et al found the risk of cardiovascular events increased with increasing carotid IMT (195), but Den Ruijter found that when carotid IMT was added to traditional cardiovascular risk models the added value was small and not thought to be of clinical importance (194).

There have been contradictory results not only regarding the value of carotid ultrasound in cardiovascular risk prediction, but also in assessing cardiovascular risk in women with pre-

eclampsia. This may be partly due to problems such as varying study sizes and varying carotid ultrasound protocols. There can be differing results depending on which segments of the carotid artery are imaged and whether carotid plaque assessment is included (186). Carotid plaque presence has been found to have a stronger effect on improving cardiovascular risk prediction in women rather than men (196).

A recent systematic review and meta-analysis by Milic et al (197) found that women who had pre-eclampsia had a higher carotid IMT than normotensive women at time of diagnosis and within the first decade postpartum. This is during the period of time when the majority of women are still pre-menopausal. The onset of menopause is also associated with an increase in cardiovascular risk factors, as mentioned in a study by Johnson et al (198). In this study, among healthy women undergoing repeated carotid IMT measurement over a 3 year period, a higher rate of preclinical cardiovascular disease progression was noted in women with a more rapid menopausal transition (198). In order to assess carotid IMT and subclinical atherosclerosis in women with more remote histories of pre-eclampsia, at ten or more years since pre-eclamptic pregnancy, Garovic et al performed a meta-analysis of these studies and presented the data which included their own most recent study (199). In their own study of women at approximately 30 years since pre-eclampsia, carotid IMT was increased in women with a history of pre-eclampsia in comparison with normotensive pregnancies, and this association was independent of other cardiovascular risk factors (199). The meta-analysis of women with pre-eclampsia 10-40 years ago showed a similar difference; women with a history of pre-eclampsia had higher carotid IMT measurements than those with normotensive pregnancies (199).

1.5 **Cardiological findings in pre-eclampsia**

It has long been acknowledged that pregnancy may act as an early “stress test” and identify women at higher risk of cardiovascular disease later in life (79). An imbalance between pro-angiogenic vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) and anti-angiogenic soluble Fms-like tyrosine kinase 1 (sFlt-1) contributes to pre-eclampsia as discussed further in section 1.7.1. The fact that PlGF is expressed in cardiac and lung tissue as well as the placenta may be important in the evolution of cardiac problems in pre-eclampsia (200), and cardiac findings in relation to pre-eclampsia are further explored in this section.

1.5.1 **Echocardiography**

One in three women with a history of pre-eclampsia will develop hypertension within 12 years of delivery (201). Evidence is mounting that women with pre-eclampsia can develop abnormal left ventricular geometry and decreased diastolic function which may persist (202;203). Normally in pregnancy there is an increase in left ventricular (LV) mass and its dimensions also increase. It is an eccentric hypertrophy which is a physiological process and reverses within a few weeks post-partum (203). It is a more concentric hypertrophy which develops in pre-eclampsia and this is as a consequence of the increase in LV workload caused by the higher blood pressure (204). It is possible that excessive cardiac remodelling does not revert back to normal after delivery (201).

A recent study by Melchiorre et al (202) found that at 1 year post-partum, asymptomatic left ventricular moderate-severe dysfunction/hypertrophy was significantly higher in women who had experienced pre-term pre-eclampsia in comparison with term pre-eclampsia or matched controls. This study also found that the risk of developing essential hypertension within 2 years post-partum was significantly higher in pre-term pre-eclamptic women and women with persistent LV moderate /severe abnormal function (202). In a large multi-centre echocardiographic study by Scantlebury et al (205), a history of hypertensive pregnancy was associated with left ventricular hypertrophy after adjusting for traditional risk factors.

In a study focusing on whether women with persistent cardiac dysfunction after pre-eclampsia were at risk of recurrent pre-eclampsia (206), Valensise et al found that women with recurrent pre-eclampsia compared with controls and non-recurrent pre-eclampsia had lower stroke volume, lower cardiac output and higher total vascular resistance. In both the recurrent and non-recurrent pre-eclampsia groups left ventricular mass index was higher than in the control group. There was also progressive impairment of diastolic function in women with a history of pre-eclampsia, and those with recurrent pre-eclampsia were affected worse than those with non-recurrent pre-eclampsia (206). Echocardiography could therefore be a useful tool in evaluating cardiovascular risk after pre-eclampsia, and merits further consideration.

1.5.2 **The Electrocardiogram**

American Heart Association Electrocardiography and Arrhythmia Committee made recommendations in 2009 for the interpretation of the electrocardiogram including the ST segment, T and U waves and the QT interval (207). The QT interval is defined as the interval from the onset of the QRS complex to the end of the T wave and should be measured in the lead showing the longest QT interval (207) which tends to be V2 or V3 (see Figure 1.7). The QT interval varies depending on heart rate, with a shorter QT interval with a faster heart rate and a longer QT interval with a slower heart rate (208). Several different methods of "correcting" the QT interval have been devised and they work out the expected value, the corrected QT (QTc) interval, if the heart rate was 60 beats per minute. The most popular methods of QT correction are Bazett's, Fridericia, Framingham and Hodges (208). Both Bazett and Fridericia formulas are nonlinear and Framingham and Hodges are linear functions. While Bazett's correction is the most commonly used method, the results of a study by Luo et al (208) revealed that it did not perform as well as other methods. For example, the study analysed 10,303 ECGs and found that 30% of apparently normal ECGs would be reported by Bazett's as having abnormal QT intervals for a 440ms threshold, and 10% if a 460ms threshold was used. The other formulae would have been incorrect in <2% of the ECGs (208). The study concluded that Hodges QTc formula performed the best.

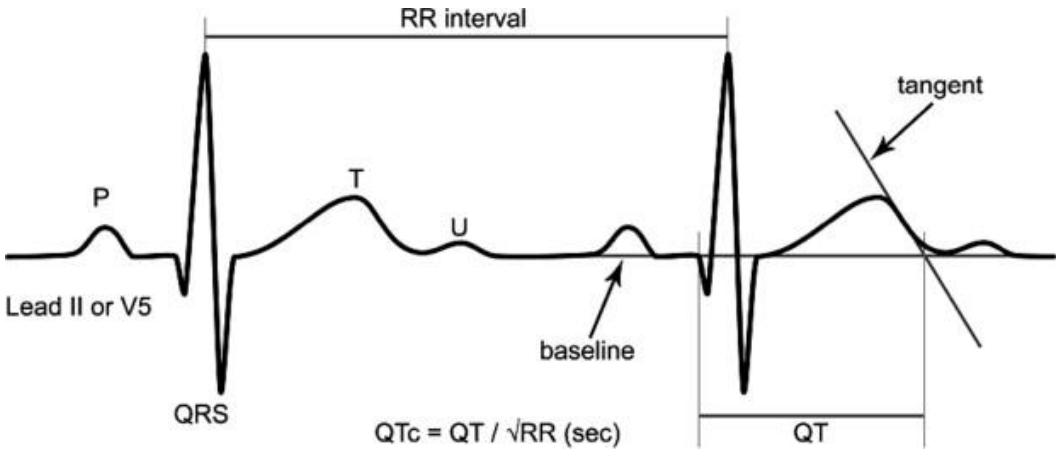


Figure 1.7 Method for measurement of the QT interval. A tangent is drawn to the steepest slope of the last limb of the T wave. The end of the T wave is the intersection of the tangent with the baseline. From Postema et al (209) with permission.

Minnesota code classifications are widely used in clinical studies to describe features of the electrocardiogram. These codes provide a standardised, objective method of ECG analysis and have proven to be extremely useful (210).

Little is known about the ECG during pregnancy and the post-partum period. The best method of QT interval correction has not been ascertained and accurate measurement is particularly important concerning the increased risk of arrhythmia and death associated with a prolonged QT interval. Also there are limited studies investigating the ECG both during pregnancy and the post-partum period. Pre-eclampsia may be a risk factor not only for future cardiovascular disease, but also for arrhythmia (200).

Normal adaptations to pregnancy include increase in heart rate, which reaches a peak in the third trimester. Gestational age impacts on the QRS complex and T waves by promoting a leftward axis shift as pregnancy progresses. In the majority of women there is a leftward QRS axis shift during the second and third trimesters which changes to rightward before delivery (211). There is evidence that hypertensive disorders of pregnancy are associated with changes in P-wave morphology and QT interval. Isezuo and Ekele showed that eclampsia was associated with prolonged ventricular repolarisation (212). Raffaelli et al more recently published a study looking at 76 women affected by pre-eclampsia and a control group of pregnant women without cardiovascular disease or gestational hypertension (213). All participants had a 12-lead ECG performed prior to delivery. Pre-eclamptic women had a lower heart rate, a longer P-wave duration, a longer mean corrected QT interval (QTc) and a higher QT dispersion. QT dispersion is defined as the difference between the maximum and minimum QT interval measured in each ECG lead. Prolongation of QT dispersion is correlated with an increased incidence of ventricular arrhythmias and is a known predictor of all-cause mortality (214). It has also been found that non-specific changes in the ECG can predict morbidity and mortality due to cardiovascular disease in the future and it is used in risk estimation and screening in the hypertensive population (215;216).

In a controlled cross-sectional study the ECGs of 64 women with pre-eclampsia were compared with 32 healthy women with uncomplicated pregnancies in the third trimester. QT parameters, the interval between the peak and end of the T wave (Tp-e) , and Tp-e/QT ratio were compared and Tp-e interval and Tp-e/QT ratio were significantly higher in pre-eclampsia (200).

In normal pregnancy the heart rate increases and peaks during the third trimester (211). There is generally reported to be a leftward shift in the QRS-axis as pregnancy progresses and in particular during the 2nd and 3rd trimesters. Before delivery there is a rightward axis shift in most women. However some previous studies have shown conflicting results. The QTc interval was thought not to be significantly altered in normal pregnancy (211), however two recent studies have found significantly different QTc intervals in pregnant women vs non-pregnant women, with a longer QTc interval noted in pregnancy (although still within the normal range).

In a large retrospective cohort study by Ray et al, women with maternal placental syndromes including pre-eclampsia were found to be at a higher risk of premature heart failure and dysrhythmias, starting one year after delivery (217). Monitoring of these women to facilitate early intervention and avoid serious consequences is important to consider.

1.6 **Renal consequences of pre-eclampsia**

In normal pregnancy the kidneys can increase in size by up to 30%, with 1-1.5cm increase in length (218). Renal function itself changes in response to hormonal variations during the menstrual cycle and the mean arterial pressure and systemic vascular resistance are lower in the mid-luteal phase than the mid-follicular phase. This results in an increase in cardiac output, renal plasma flow (RPF) and glomerular filtration rate (GFR) (218). These changes continue in pregnancy and at term GFR has been found to be 40% higher in comparison with non-pregnant women, but returns to normal 1 month after delivery (219).

Pre-eclampsia, HELLP syndrome, eclampsia and acute fatty liver of pregnancy (AFLP) have considerable overlap in presentation and may all share a similar pathophysiology. Pre-eclampsia, eclampsia or HELLP syndrome can develop from mild to severe microangiopathy affecting the placenta, liver, kidneys and brain. A decline in renal function develops and in the developed world, severe pre-eclampsia accounts for ~40% of pregnancy-related acute kidney injury (218).

Microalbuminuria is itself a risk factor for cardiovascular events (220). An association between end stage renal disease (ESRD) after pre-eclampsia has also been shown in a

Norwegian study of 570,433 women (221). The study population consisted of women who had a first singleton birth between 1967 and 1991. Pre-eclampsia during the first pregnancy was associated with a relative risk of ESRD of 4.7 (95% confidence interval [CI], 3.6 to 6.1). In women with two or more pregnancies, pre-eclampsia during the first pregnancy was associated with a relative risk of ESRD of 3.2 (95% CI, 2.2 to 4.9), during the second pregnancy with a relative risk of 6.7 (95% CI, 4.3 to 10.6), and during both pregnancies with a relative risk of 6.4 (95% CI, 3.0 to 13.5). Among women who had been pregnant three or more times, pre-eclampsia during two or three pregnancies was associated with a relative risk of 15.5 (221). It is clear, from this and other studies, that ESRD has associations with pre-eclampsia (221-223). However the precise mechanism of this increased risk of renal disease is not well understood (224).

A further complication to consider regarding the renal system is renal conditions which may worsen during pregnancy and which may mimic pre-eclampsia. For example, pre-eclampsia and chronic kidney disease may both present during pregnancy with worsening hypertension and proteinuria (224). Women with lupus nephritis during pregnancy may also present with proteinuria, hypertension and increased creatinine (224). In order to better differentiate between these renal diseases and pre-eclampsia, the role of biomarkers as a potential tool has been studied. In one such study (225) maternal serum levels of sFlt-1 were much higher and PlGF levels much lower in women with pre-eclampsia in comparison with chronic kidney disease patients or women with normotensive pregnancies. In a small study investigating systemic lupus erythematosus (SLE) and pre-eclampsia, women with both pre-eclampsia and SLE had a significantly higher serum sFlt1 concentration than women with SLE but no pre-eclampsia (226).

1.7 **Biomarkers in pre-eclampsia**

There are a number of circulating biomarkers which are associated with pre-eclampsia and this section further describes them and their role in cardiovascular disease.

1.7.1 **Markers of angiogenesis**

Markers described in this section are involved in angiogenesis, vasculogenesis and play a crucial role in normal placental development. Two pro-angiogenic growth factors, vascular

endothelial growth factor (VEGF) and placental growth factor (PlGF) are important in trophoblast proliferation and the normal process of implantation (227).

In normal pregnancy levels of pro-angiogenic placental growth factor (PlGF) peaks at around 30 weeks (228), decreasing towards term (229). In pre-eclampsia levels of circulating PlGF are reduced, particularly in cases of early-onset pre-eclampsia (230;231). In pre-eclampsia, levels of VEGF are lower than in normal pregnancy and VEGF antagonists which are used in patients with cancer can produce glomerular endothelial damage, hypertension and reversible posterior leucoencephalopathy, which are similar to findings in pre-eclampsia and eclampsia (232;233).

A splice variant of the vascular endothelial growth factor receptor Flt-1 (sFlt-1) is a circulating anti-angiogenic protein. It inhibits the pro-angiogenic VEGF and PlGF (19). sFlt-1 is made in the placenta in the syncytiotrophoblast layer before being secreted into the maternal circulation (19). In normal pregnancy maternal concentrations of the anti-angiogenic protein soluble fms-like tyrosine kinase-1 (sFlt-1) do not change throughout the first two trimesters, increasing throughout the third trimester (229). Even though the placenta is the main source of sFlt-1, the major site of its production is thought to be syncytial knots which are degenerating syncytiotrophoblast tissue (234) and this is increased in pre-eclampsia.

In pre-eclampsia there is an increased level of sFlt-1 in the second and third trimesters (228). The levels increase before onset of clinical symptoms (229;235). As sFlt-1 levels increase, PlGF and VEGF decrease. In mouse models of pre-eclampsia, the phenotype can be improved by antagonising sFlt-1 (236).

Soluble endoglin (sEng) is also anti-angiogenic by disrupting transforming growth factor-β (TGFβ) signalling in vasculature (237). Endoglin is a glycoprotein which is expressed on endothelial cells and placental syncytiotrophoblasts (228). It may also play a role in the pathophysiology of pre-eclampsia (19). As levels of sFlt-1 and sEng increase and PlGF decreases in women with pre-eclampsia, they correlate with disease severity and with gestational age (229;238-240). When sFlt-1 and sEng are administered to pregnant rats, a pre-eclampsia-like phenotype is produced with glomerular endotheliosis, proteinuria, hypertension and fetal growth restriction and thrombocytopenia (241).

The potential for angiogenic markers to be used in the prediction of pre-eclampsia is an ongoing area of research. For example in a prospective multicentre observational study Zeisler et al sought to derive and validate a cut-off value for the sFlt-1/PlGF ratio for which levels at or below the cut-off would predict the absence of pre-eclampsia one week after the visit, and levels higher than the cut-off would predict pre-eclampsia developing within 4 weeks (242). A cut-off value of 38 was found to have important predictive value in separate development and validation cohorts. The utility of such a test, in accurately predicting the absence of disease early on, would assist greatly with management decisions in pregnant women (e.g. home vs hospital in women with possible pre-eclampsia).

With regard to the role of markers of angiogenesis in cardiovascular disease there is considerable evidence in the literature. In patients with acute coronary syndrome, PlGF has been found to be upregulated in atherosclerotic lesions (243). It is thought to have an inflammatory role in atherosclerotic plaque instability. Increased circulating levels of PlGF may be representative of a greater risk for cardiovascular events and mortality in patients with heart disease. PlGF levels have also been found to positively correlate with C-reactive protein (CRP) and it is hypothesised that the source of increased PlGF is inflamed endothelium (243). VEGF and sFlt-1 have also been associated with cardiovascular disease (244;245). VEGF is known to be increased in patients with cardiovascular risk factors such as hypertension, atherosclerosis and hyperlipidaemia (244;246;247). sFlt-1 correlates with severity of cardiovascular disease (248) and is independent of other risk factors. sEng has been implicated in hypercholesterolaemia and endothelial dysfunction (249).

In women with a history of hypertensive disorder of pregnancy, studies of angiogenic markers have been evaluated in two recent systematic review and meta-analyses (179;250). Visser et al (250) reported higher median VEGF levels in women with a history of hypertensive pregnancy, however, only two studies had reported VEGF levels (251;252). One of these studies reported a higher mean sFlt-1 level (252) and the other, a higher median sFlt-1 (251). PlGF and sEng were not evaluated in this paper. The other meta-analysis by Grand'Maison et al (179) also evaluated VEGF and sFlt-1. It found that on pooled analysis, there was no significant difference in levels of VEGF between women with a history of hypertensive disorder of pregnancy and those with normotensive pregnancies. Mean levels of sFlt-1 were higher in women with a history of hypertensive disorder of pregnancy.

1.7.2 **Markers of inflammation**

1.7.2.1 ***Cytokines***

Some of the more commonly researched cytokines with regard to pre-eclampsia are interleukin-6 (IL-6), interleukin-10 (IL-10) and tumour necrosis factor-alpha (TNF-α). IL-10 is an anti-inflammatory product of the T-helper 2 (Th2) cells (253), IL-6 is produced by the placenta, vascular endothelial cells and leucocytes and is a multifunctional cytokine (254) and TNF-α is mainly activated by macrophages and monocytes and stimulates the release of other cytokines (254).

The maternal systemic inflammatory response is considered to be enhanced in pre-eclampsia (254). Plasma levels of TNF-α and IL-6 have been found to be raised in pre-eclampsia (254-257). IL-10 levels have also been found to be higher in pre-eclampsia (258).

IL-6 has been implicated in cardiovascular diseases including unstable angina and heart failure (259-263). IL-10 has also been implicated in cardiovascular disease with several studies showing contradictory findings (264-268). In addition, studies of TNF-α have reported conflicting roles such as beneficial effects in myocarditis, pressure overload and cardioprotection against ischaemia but possible adverse effects from atherosclerosis and reperfusion injury to heart failure and hypertrophy (269).

In women with a history of hypertensive pregnancy, studies have revealed both higher and lower TNF-α levels (250;256;270;271) and a study by Girouard et al (270) reported higher IL-6 levels in women with a history of hypertensive disorders of pregnancy. Freeman et al reported higher IL-6/IL-10 ratio in women with a history of pre-eclampsia (256).

1.7.2.2 ***Adhesion Molecules***

Cell adhesion molecules promote attachment of leucocytes to the endothelium, to sites of inflammation and facilitate their movement in arterial walls (272). An increase in soluble vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) is thought to be indicative of endothelial cell activation (273). An increase in soluble P-selectin in thought to represent platelet activation and an increase in soluble E-

selectin is thought to be a marker of endothelial cell activation or dysfunction. Differences in soluble L-selectin are thought to represent leucocyte activation (273). In pre-eclampsia plasma levels of E-selectin, P-selectin, ICAM-1 and VCAM-1 have been found to be increased, and levels of L-selectin decreased (273;274).

In cardiovascular disease there is evidence that soluble cell adhesion molecules may play a role (272). For example, using "Atherosclerosis Risk In Communities (ARIC)" Study participants, Hwang et al (275) found higher ICAM-1 and E-selectin levels in subjects with coronary heart disease and coronary artery atherosclerosis in comparison with control subjects however levels of VCAM-1 were not significantly different (275). Luc et al (276) investigated ICAM-1 and VCAM-1 in relation to myocardial infarction and coronary death or angina in a group of healthy men aged 50-59 years. ICAM-1 was found to be predictive of myocardial infarction or death and angina, however VCAM-1 was not associated with the occurrence of a first cardiovascular event (276). Nevertheless, a recent study by Kunutsor et al (272) has revealed some unexpected findings regarding VCAM-1 in comparison with previous literature. The study consisted of a large cohort of 2,638 participants without any cardiovascular disease at baseline, from the "Prevention of Renal and Vascular End-stage Disease (PREVEND)" study. An inverse association between VCAM-1 and cardiovascular disease was found after adjusting for cardiovascular risk factors.

In assessing adhesion molecules in women with a history of hypertensive pregnancy disorders, the meta-analysis by Visser et al (250) revealed that five studies found no difference in mean ICAM levels and three studies showed no difference in mean VCAM levels between women with a history of hypertensive disorders of pregnancy and women with a normal pregnancy. One study described higher median ICAM and VCAM levels (277). Another meta-analysis by Grand'Maison et al (179) revealed that on pooled analysis, neither ICAM nor VCAM levels were different between women with a history of pre-eclampsia and those with normotensive pregnancies.

1.7.2.3 ***CRP***

C-reactive protein (CRP) is an acute phase protein produced in the liver in response to inflammatory stimuli (278). CRP has also been found in amniotic fluid (279). In a recent study by Parchim et al (280) the placenta was identified as a site of production of CRP. In

normal pregnancy an increased CRP level has been noted (281), however, in pre-eclampsia CRP levels are even higher. Previous studies have proposed that pre-eclampsia is related to CRP, and a meta-analysis (278) revealed a significant relationship however body mass index was found to be an important modifier.

CRP has been linked with cardiovascular disease and has been implicated in endothelial damage by increasing foam cell formation, leading to atherosclerosis (280;282). It has been associated with renal dysfunction and CRP also correlates with kidney function decline in patients with chronic kidney disease (280). This is thought to be related to increased oxidative stress from the deposition of CRP in the glomeruli.

In a study by Hubel et al (283), significantly elevated CRP levels have been found in women 30 years after pregnancy complicated by eclampsia after adjustment for age, smoking, body mass index and hormone replacement.

1.7.3 **Renin-Angiotensin Aldosterone system**

The renin-angiotensin-aldosterone system (RAAS) is involved in the regulation of renal cardiac and vascular physiology. It is implicated in many diseases including renal disease, hypertension and heart failure, and activation of RAAS can result in vasoconstriction, fibrosis, and vascular and cardiac hypertrophy (284).

Renin is synthesized by the juxtaglomerular cells of the kidney and is secreted in response to lower than normal renal perfusion or low sodium concentration. Renin then cleaves angiotensinogen, which is made in the liver, to angiotensin I. This in turn is transformed into angiotensin II by angiotensin-converting enzyme (ACE), in the lung (284). Angiotensin II is a powerful vasoconstrictor and influences the release of aldosterone from the renal cortex. Aldosterone exerts its effect on blood volume and blood pressure by adjusting reabsorption of sodium and excretion of potassium in the kidney.

In normal pregnancy generalised vasodilation may result in activation of the RAAS to enable sodium and water retention (285). In normotensive pregnancies this leads to increased levels of renin, angiotensin II and aldosterone (286). However, during pregnancy there are components of the RAAS which can be synthesized by the placenta (286). Higher than expected levels of aldosterone are found (286) and placental size and trophoblast

growth have been found to be positively correlated with the presence of aldosterone (287). Features of intrauterine growth restriction (IUGR) are present in animal models with reduced aldosterone secretion (288). Another interesting feature of the RAAS in normal pregnancy is that women do not appear to respond to the vasoconstrictor effect of angiotensin II and blood pressure remains low despite the observed increase in angiotensin II (286).

One possibility for the increased expression of aldosterone in pregnancy is angiotensin II-independent production of aldosterone (287). A recent study by Gennari-Moser et al revealed VEGF to be more powerful than angiotensin II in stimulating adrenal aldosterone (289). Aldosterone levels are lower in pre-eclamptic pregnancies than normotensive pregnancies (286). In a rat model, sFlt-1 (the VEGF inhibitor) resulted in a fall aldosterone concentration, and might explain the low levels of aldosterone in pre-eclampsia (286;289).

Also of great relevance is the fact that a higher frequency of women with pre-eclampsia have been found to have loss-of-function mutations in the gene for aldosterone synthase *(CYP11B2)* (290). A lower frequency of gain-of-function mutations in this gene have been found in pre-eclampsia (291). Studies in women with a history of hypertensive disorders of pregnancy have revealed that these women have an increased sensitivity to angiotensin II (292) at ≥8 months post-partum, and an experimental rat model has revealed an increase in responsiveness to angiotensin II following pre-eclampsia (293). The animal model in particular supports the argument that pre-eclampsia itself may influence cardiovascular disease risk.

Further research into the complexities of the RAAS in pregnancy, pre-eclampsia and its potential role in future cardiovascular risk after hypertensive disorders of pregnancy is warranted.

1.7.4 **Uric acid**

Hyperuricaemia may directly contribute to vascular damage and hypertension (294;295) and is therefore important in cardiovascular risk.

In pre-eclampsia the elevation in uric acid tends to precede the onset of proteinuria and fall in GFR (26;296). The degree of uric acid elevation not only correlates with maternal

morbidity (297) and severity of proteinuria (298) and renal changes (299), but it has also been found to correlate with fetal mortality (300). The mechanism of uric acid elevation in pre-eclampsia is unresolved but may be due to decreased renal clearance or tissue injury/ischaemia (26).

In women with hypertensive disorders of pregnancy, these higher uric acid concentrations have been found to persist even decades after the pregnancy. In a large study by Weissgerber et al of 2472 women from 1282 sibships (301) women who had a history of hypertensive disorders of pregnancy were more likely to have uric acid concentrations >5.5mg/dL than women with normotensive pregnancies. These differences persisted after correcting for traditional cardiovascular risk factors and co-morbidities. Family-based subgroup analysis which compared women with hypertensive pregnancies vs their parous normotensive sisters also revealed a significant difference in uric acid concentrations between the two groups. Therefore, uric acid concentrations were found to be higher decades after pregnancy, however familial pre-disposition did not explain these findings. Several smaller studies have suggested uric acid concentrations are increased or not different in women with a history of pre-eclampsia or other hypertensive disorders of pregnancy in comparison with normotensive pregnancies (137;143;302). These conflicting results merit further investigation, especially considering the role hyperuricaemia may play in cardiovascular disease.

1.7.5 **Homocysteine**

Homocysteine has primary atherogenic and pro-thrombotic properties and has been linked to coronary heart disease previously (93;303). It is a metabolite of the amino acid methionine and homocysteine levels are influenced by several different mechanisms ranging from intake of folate and cobalamin to genetic polymorphisms in the *MTHFR* gene (304) which are discussed more fully below. Homocysteine is associated with arterial wall inflammation and myocardial infarction and has been found to have an effect on cardiovascular risk above that of inflammatory markers and traditional cardiovascular risk factors (305). Vitamin B12, folate and vitamin B6 are necessary for homocysteine metabolism (306).

The human *MTHFR* gene on chromosome 1p36.3 encodes methylenetetrahydrofolate reductase (MTHFR). This enzyme catalyses the reduction of 5, 10-

methylenetetrahydrofolate to 5-methyltetrahydrofolate (307). Folate and total homocysteine (tHcy) levels can be influenced by the presence of C677T and A1298C mutations in the *MTHFR* gene (307). Women with these mutations have higher levels of plasma homocysteine in comparison with women who have a normal genotype. For example, the C677T substitution causes abnormalities of folate binding and reduced MTHFR enzyme activity (307). There is an increased folic acid requirement in order to sustain usual homocysteine remethylation to methionine. Overall the *MTHFR* C677T mutation is associated with a slightly increased plasma tHcy concentration and a lower folate level in red blood cells, serum and plasma (307).

Levels of homocysteine have been found to be elevated in women with a history of hypertensive disorders of pregnancy (250;304). More specifically, pre-eclampsia has been associated with hyperhomocysteinaemia (308;309). It is possible that the pathophysiology of pre-eclampsia may involve the *MTHFR* C677T mutation. Previous studies have been contradictory and results from a meta-analysis by Wu et al (307) revealed that *MTHFR* C677T was associated with risk of pre-eclampsia, especially in Caucasians and Asians, but *MTHFR* A1298C was not (307).

A systematic review and meta-analysis by Visser et al (250) found that homocysteine levels were higher in women with disorders of hypertensive pregnancies in comparison with women with normotensive pregnancy. A study by Vollset et al investigated the relationship between pre-eclampsia and plasma homocysteine levels (310). The adjusted risk for pre-eclampsia was 32% higher in women with plasma homocysteine levels in the upper quartile in comparison with women with plasma homocysteine levels in the lower quartile. However, a later study by Timmermans et al revealed no effect of folic acid on the occurrence of gestational hypertension or pre-eclampsia (311). When homocysteine was included as part of an early prediction model in a study by Masoura et al (306) it was found that in the first trimester of pregnancy, elevated levels of homocysteine combined with higher values of other biomarkers could predict hypertensive disorders of pregnancy at a later gestation.

A study by Wang et al (312) found that folic acid supplementation and higher dietary folate intake during pregnancy reduced the risk of pre-eclampsia and that the risk reduction may vary depending on severity of pre-eclampsia. A prospective cohort study by Wen et al (313) assessed the effect of folic acid supplementation in pregnancy on the risk of pre-

eclampsia and found that the rate of pre-eclampsia was lower in women on folic acid supplementation, and the difference was statistically significant in women with a high risk of pre-eclampsia. High risk pregnancy was classified as previous history of pre-eclampsia, chronic hypertension, diabetes, multiple pregnancy and body mass index $\geq 35 kg/m^2$. A Cochrane review of homocysteine-lowering interventions for preventing cardiovascular events (314) did not find any differences between supplements of vitamins B6, B9 or B12 (given in combination or alone) and placebo in myocardial infarction, death from any cause or adverse events.

Further studies are required to clarify the part homocysteine plays in the pathophysiology of pre-eclampsia and the possible role of folic acid in future management of pre-eclampsia.

1.7.6 **Lipids**

High serum lipid levels are known to contribute to atherosclerosis (315;316). In particular low-density lipoprotein (LDL) cholesterol has been implicated (316). High-density lipoprotein (HDL) cholesterol is thought to be inversely related to cardiovascular risk (317). This decrease in risk is thought to be due to the role of HDL in removing excess cholesterol from the peripheral tissues and transporting it to the liver to be excreted in bile (318). HDL cholesterol has other cardio-protective properties such as the inhibition of inflammation and it has anti-thrombotic properties and can promote endothelial repair (318).

In normal pregnancy cholesterol is necessary for the synthesis of placental steroid. Levels of total cholesterol, HDL cholesterol, triglycerides and LDL cholesterol increase (319). During the first two trimesters maternal fat stores accumulate as a source of calories for later in pregnancy and the post-partum period (319;320). Anabolic metabolism and hyperphagia aid the increase in maternal adipose tissue stores (321). In the third trimester, maternal metabolism becomes catabolic, and by late pregnancy there is peripheral adipose tissue lipolysis and increasing maternal insulin resistance (321). Maternal free fatty acids and lipoprotein triglyceride content are increased (321).

In pre-eclampsia there is increased maternal lipid oxidation (321) and this process may be implicated in the placental vascular dysfunction and endothelial dysfunction which characterises the condition. In the placenta lipid-filled foam cells have been found to

accumulate in the spiral arteries as part of a process of acute atherosis (322). This process is not pathognomonic of pre-eclampsia and can be seen in other conditions such as diabetes, thrombotic conditions and intrauterine growth restriction (322).

A recent meta-analysis of studies investigating the relationship between pre-eclampsia and maternal serum triglycerides revealed higher triglyceride levels in women with a history of pre-eclampsia in comparison with normotensive women (319;323). In a systematic review and meta-analysis examining pregnancy levels of total cholesterol, LDL cholesterol, high-density lipoprotein (HDL) cholesterol, non-HDL cholesterol (e.g. very low density lipoproteins (VLDL)), and triglycerides and subsequent pre-eclampsia risk, maternal serum total cholesterol, non-HDL cholesterol and triglycerides were higher during all trimesters in women who developed pre-eclampsia (319). There were also increased levels of LDL cholesterol in all trimesters in women who developed pre-eclampsia in comparison with normotensive controls but the results were of borderline significance (319). HDL cholesterol was lower during the third trimester in women with pre-eclampsia in comparison with controls (319). Increased triglyceride in cells is associated with reduced prostacyclin release, and raised triglycerides have also been linked to alterations in LDL particle size, with a shift to smaller more atherogenic subtypes (318).

New insights into the potential role of obesity in the risk of pre-eclampsia have recently been described by Huda et al (324). This group hypothesised that adipocyte release of pro-inflammatory adipokines is exaggerated under basal and stressed conditions in pre-eclampsia in comparison with controls. They also hypothesized that adipose tissue macrophage infiltration is increased in women with pre-eclampsia, and that this response might be more obvious in a particular type of adipose tissue e.g. visceral in comparison with subcutaneous. Macrophages are important mediators of inflammation in adipose tissue and promote insulin resistance in white adipose tissue (324). sFlt-1 has been shown to be secreted by adipocytes in the non-pregnant state, and sFlt-1 production by adipocytes in pre-eclampsia had not previously been investigated (324). They found that visceral adipose tissue in pre-eclampsia had higher activated macrophage content and higher expression of TNFα than controls. They also found that adipocytes from visceral adipose tissue in pre-eclampsia was more responsive to lipopolysaccharide stimulation in that they released higher levels of IL-6 and TNFα (324). In pre-eclamptic cases there was no significant evidence of sFlt-1 release from adipocytes, which suggests adipocytes do not act as an additional source of sFlt-1 in pre-eclampsia (324).

The menopause transition brings with it changes in the lipoprotein subclass profile, but the postmenopausal increased risk of cardiovascular disease may also be caused by more vulnerable vessels due to adipose tissue re-distribution, decreased oestrogen and aging (325). Oestrogen has LDL-lowering effects, however this does not fully explain the pattern of cardiovascular risk in women (316). In the Framingham heart study, women with incident coronary heart disease had increased triglyceride levels and decreased HDL levels (326). When elevated triglyceride levels occur in conjunction with increased serum LDL, the risk of cardiovascular disease is increased above that of raised LDL without hypertriglyceridemia (316;327). Other actions of hypertriglyceridemia are an increase in glucose intolerance and insulin resistance, decreased HDL cholesterol, prothrombotic states and hypertension. These actions have a compound effect on cardiovascular risk (327).

Studies of lipids in women with a remote history of pre-eclampsia have also provided some important insights. An Icelandic study of postmenopausal women after eclampsia found that in comparison with women who experienced normotensive pregnancies, a greater number were on hypertensive medication, and they had increased levels of apolipoprotein B, smaller-sized LDL particles and increased total cholesterol:HDL cholesterol ratio (328). Women who had eclampsia and had recurrent hypertension in pregnancy also had much smaller LDL sizes in comparison with women who had non-recurrence (328). A meta-analysis has suggested that non-HDL cholesterol levels such as VLDL and apolipoprotein B-containing lipoproteins may be even better than LDL cholesterol levels at predicting cardiovascular disease (329). In a more recent large prospective cohort study (330), women who were 18 years postpartum with a past diagnosis of pre-eclampsia or gestational hypertension had higher triglycerides and lower HDL cholesterol levels than women with normotensive pregnancies.

Statins have been avoided in pregnancy in the past, due to concerns regarding teratogenesis (321). Nevertheless, their actions on lipids and endothelial function and their anti-inflammatory and anti-oxidant properties have made them an attractive prospect in the potential treatment of pre-eclampsia (321). Studies such as the “Statins to Ameliorate early onset Pre-eclampsia (StAmP)” are further evaluating their role (331;332).

1.7.7 **Proteomics**

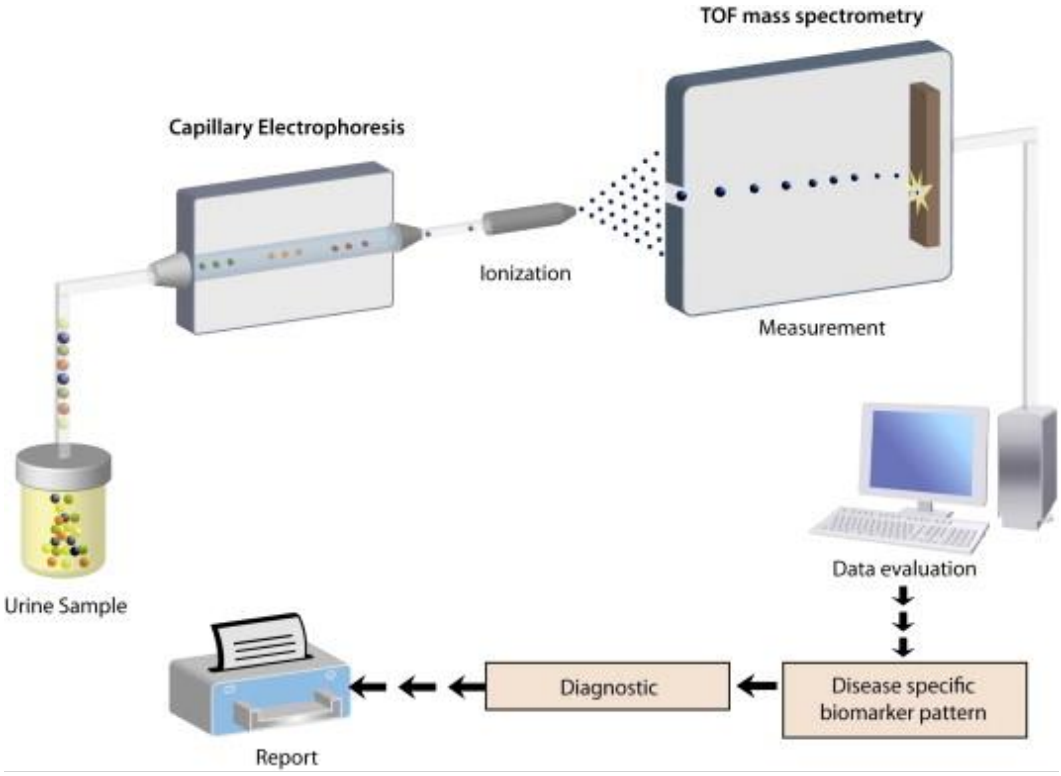


Figure 1.8 Capillary electrophoresis (CE) coupled to mass spectrometry (MS). Proteins and peptides are separated by capillary electrophoresis, ionised by electrospray ionization (ESI) and detected in a time-of-flight mass spectrometer (TOF-MS). Figure reproduced with permission from Raedler et al (333).

Proteomics is the study of the proteome, which reflects the protein content of the genome (334). The proteome refers to the whole protein content of the cell, tissue, organism or different bodily fluids. Proteome profiles change depending on variations in gene expression, alternative splicing, and post-translational modifications (335).

It is a rapidly advancing field, and with the development of high-throughput techniques it is hoped that this might facilitate the discovery of even more biomarkers implicated in the pathophysiology of disease and with the potential for therapeutic targeting. Specific protein biomarker panels for several different diseases have already been identified.

Proteomic analysis can be performed on any type of human tissue, bodily fluid or cultured cells. Urinary proteins have been found to remain stable for several years without

significant alterations in the proteome, even at temperatures as low as -20^{o}C (336), which is a desirable quality in research. Urine is available in large volumes and collection is non-invasive. Also the simplicity of sampling make it convenient for collection in the research and clinic setting.

There are several methods of proteomic analysis available including liquid chromatography coupled to mass spectrometry (LC-MS), surface-enhanced laser desorption/ionization coupled to mass spectrometry (SELDI-MS) and capillary electrophoresis coupled to mass spectrometry (CE-MS). Each has advantages and disadvantages. LC-MS is multidimensional and high sensitivity but is time-consuming with a restricted mass range (336). SELDI-MS is easy to use and requires a low sample volume but is restricted to selected proteins, has low information content and lack of comparability (336). CE-MS is high-sensitivity and fast, with low sample volumes and is low cost but is not suited to larger proteins (>20kD) (336).

Capillary electrophoresis (CE) which was used for the proteomic analyses performed in this thesis separates proteins based on their migration through a buffer-filled capillary. Separated proteins are then delivered from the end of the capillary by nano-ion spray into a mass-spectrometer (MS) (Figure 1.8).

A study by Buhimschi et al (337) sought to identify women with pre-eclampsia who would benefit from delivery based on urinary polypeptide profile of samples collected at diagnosis of the condition. This urinary polypeptide profile was able to distinguish between pre-eclampsia and other disorders causing proteinuria and other hypertensive disorders of pregnancy.

Chen et al (338) found decreased levels of SERPINA 1 in the urine of women with gestational hypertension and increased levels in pre-eclampsia. This biomarker may therefore be able to specify between some of the hypertensive conditions of pregnancy. In another study by Lee et al (339) four protein peaks were able to determine which women had severe pre-eclampsia, mild pre-eclampsia or were controls. In a study using urine samples from various time-points during pregnancy, Carty et al (340) were able to discriminate between women who proceeded to develop pre-eclampsia and matched controls. The study described a model of 50 urinary peptides which was able to diagnose

women with pre-eclampsia at 28 weeks gestation. However, the predictive value of this panel of peptides could not be verified in an independent cohort at 20 weeks gestation.

Proteomic studies require large numbers of samples in order to produce reliable results (341). Of utmost importance is the thorough phenotyping of subjects, to gain the maximum amount of information in gestational hypertensive disorders and the subtle differences between them.

1.7.8 **Genetic factors**

Some epidemiological studies have shown a strong familial predisposition to pre-eclampsia. For example, women with first degree relatives who experienced pre-eclampsia have been reported as having five times the risk of developing the condition themselves, and women with second-degree relatives affected have twice the risk (5;342). However, evidence for the placental origin of early-onset pre-eclampsia comes from the fact that parous monozygotic twins have been found to be discordant for the development of pre-eclampsia (343). An increase in risk of pre-eclampsia has also been found in women who have pregnancies with men who have a history of being involved with pregnancies complicated by pre-eclampsia in the past (5;344;345).

For most of the population, pre-eclampsia represents a complex genetic disorder occurring as the result of many common variants at different loci. Individually they have small effect, but combined they contribute to an individual's susceptibility to disease. Environmental contributions such as age and weight my also contribute to whether these variants of low penetrance may result in the disease manifesting itself (346).

Candidate gene studies focus largely on maternal genotype. The choice of gene is made based on prior knowledge of the pathophysiology of pre-eclampsia and if the gene lies within a region identified by linkage studies then this choice is further strengthened (346).

There have been a number of studies such as genome-wide scans performed to date to try to discover which genes might be involved in pre-eclampsia (347-354). Results have been limited however two susceptibility genes have been identified (*ACVR2A* and *STOX1*) (355;356). The *ACVR2A* gene codes for an Activin receptor type II which can bind Activin ligands. Activin A has a role in the placenta on both the maternal and fetal sides (356). The

STOX1 gene codes for a protein containing a winged helix domain and it acts as a transcription factor (356).

The Genetics of Pre-eclampsia (GOPEC) consortium studied 28 SNPs (in 7 genes which had previously been reported as conferring pre-eclampsia susceptibility) in 657 women and their families. None of the SNPs achieved statistical significance (357). This study re-iterated the importance of conducting studies of adequate size to provide precise genetic risks and to avoid over-reporting of false positive results (357).

In 2014 a meta-analysis study of maternal genotype and severe pre-eclampsia in 57 studies evaluating 50 genotypes (358) showed that there was a higher risk of severe pre-eclampsia with coagulation Factor V gene (proaccelerin, labile factor) *(F5)* polymorphism rs6025, coagulation factor II (thrombin) gene *(F2)* mutation G20210A (rs1799963), leptin receptor gene (*LEPR*) polymorphism rs1137100 and the thrombophilic gene group. However the meta-analysis concluded that there was potential for bias from inconsistent definition of the phenotype and poor-quality genotyping (358).

Large collaborations to ensure adequately powered studies will be an important consideration for the future. The complexities of the pathophysiology of pre-eclampsia, and the differing diagnostic criteria over time and in different countries is important to consider, especially in genetic studies where robust phenotyping will be important.

1.8 **Aims of this thesis**

The overall aim of this thesis is to explore the possible mechanisms for cardiovascular risk later in life in women with a history of pre-eclampsia.

Firstly I will study the relationship between pre-eclamptic pregnancy and cardiovascular outcomes in Scottish women using the “Generation Scotland: Scottish Family Health Study” (GS:SFHS). I will seek to confirm whether the relationship between cardiovascular risk later in life and a history of pre-eclampsia is confirmed in this population of women.

Secondly I aim to determine the effects of pregnancy on the ECG by examining the ECGs of women years after pregnancy (using GS:SFHS). Newly emerging evidence in this area suggests that women with a history of pre-eclampsia are at increased risk of cardiovascular

events and cardiovascular disease, not only through hypertension (as evidenced by a degree of ventricular hypertrophy on the ECG), but also by prolongation of the QT interval (increasing risk of arrhythmic events and sudden death).

Thirdly I aim to find out whether middle-aged women without overt cardiovascular disease, with a history of pre-eclampsia, have different vascular structure and function later on in life in comparison with those who had normotensive pregnancies.

Finally, I aim to determine whether levels of inflammatory markers, angiogenic markers, cytokines and other biomarkers are altered years after pre-eclampsia and may predispose to cardiovascular disease risk. Included are proteomic studies using previously validated panels for coronary artery disease and pre-eclampsia.

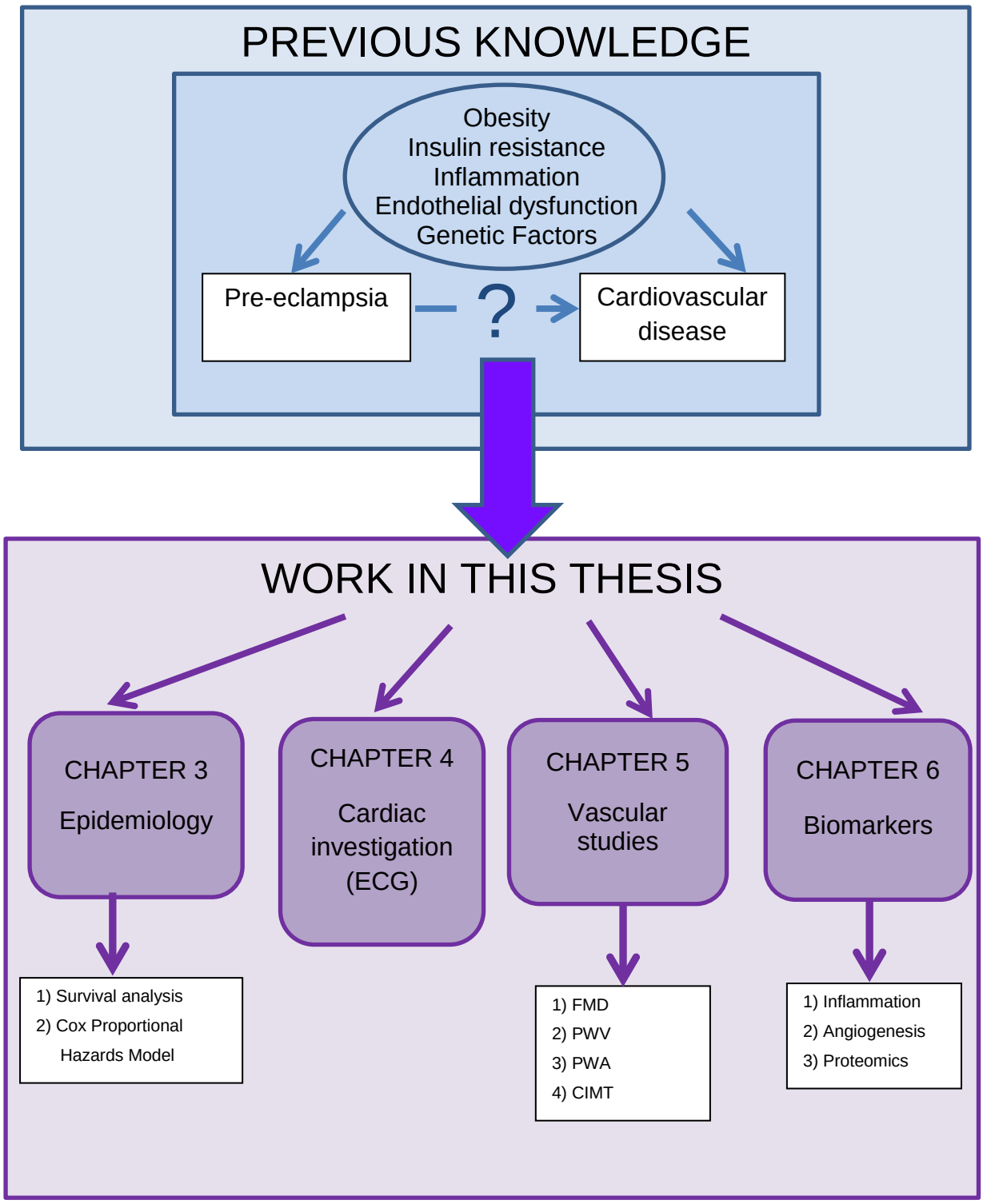


Figure 1.9. Summary of thesis

The overall aim of this thesis is to investigate the underlying mechanisms of the association between pre-eclampsia and cardiovascular disease as outlined in the flow diagram.

2 Materials and Methods

2.1 Introduction

Women with a history of pre-eclampsia are known to be at a greater risk of cardiovascular disease later in life (6), however the precise causal mechanisms of this relationship are not fully understood. The work undertaken in this thesis has aimed to address some of the key issues in further elucidating these causal mechanisms. By using a multifaceted approach of record linkage, clinical and laboratory studies, the aim was to further examine the relationship between pre-eclampsia and cardiovascular disease later in life.

The main theme of this work was to study cardiovascular risk in women 1-30 years after pre-eclampsia. First I sought to establish whether, in a relatively large cohort of parous women, there was any evidence of a greater risk of cardiovascular events in those with a history of pre-eclampsia. I then investigated whether there was any evidence of subclinical vascular damage in previously pre-eclamptic women, to determine whether there were any specific findings which might explain any increased risk. Biomarker studies were also performed to establish whether there were any differences many years after pre-eclampsia which might be in keeping with the relationship with cardiovascular disease. Finally I examined ECG data in women many years after pregnancy to identify whether there was any evidence of cardiovascular disease after pre-eclampsia.

In order to address these questions I took advantage of the Generation Scotland Scottish Family Health Study (GS:SFHS) resource. This was used for risk assessment, biomarker studies and ECG analysis. For the vascular studies I recruited my own cohort, the "Cardiovascular Consequences of Pre-eclampsia (COPS) vascular studies" cohort from women who had participated in GS:SFHS, the previous Proteomics in Pre-eclampsia study (PIP), and blood pressure clinics. This vascular study cohort was also used for additional biomarker studies.

These studies all contributed to the overall "Cardiovascular Consequences of Pre-eclampsia study" (COPS). A fuller description of individual study materials and methods are described in dedicated chapters; record-linkage study (chapter 3), ECG studies (chapter 4), vascular studies (chapter 5) and biomarker studies (chapter 6). For all studies

included in this thesis, the diagnosis of pre-eclampsia was based on ISSHP recommendations and proteinuria of at least “2+” on dipstick was used.

2.2 Funding

The COPS study was funded by the Chief Scientist Office, Scotland. Grant Ref ETM/196. The author was also funded by the European Commission’s 7th Framework Programme Collaborative Project “EU-MASCARA”.

2.3 Ethical approval

The overall COPS study (record-linkage, vascular studies and biomarker studies) was approved by the West of Scotland REC (reference 12/WS/0306) and sponsored by NHS Greater Glasgow and Clyde Research and Development Service (reference GN11CA468). Paperwork is available in Appendices 1-13. The study was registered on the UK Clinical Research Network (Ref 13723).

2.4 The Generation Scotland Family Health Study (GS:SFHS)

The GS:SFHS is a rich resource consisting of questionnaire data, clinical data and biosamples of more than 20,000 members of the Scottish general population (359;360). Study participants were recruited in Aberdeen, Dundee, Edinburgh and Glasgow between 2006 and 2011. Women from this cohort were included in the record-linkage study described in chapter 3. Clinical and ECG data which were available through the record-linkage study were further explored in chapter 4. Women who participated in GS:SFHS were also invited to attend for COPS vascular studies as outlined in chapter 5. Serum samples from the wider GS:SFHS cohort of women, stored at time of recruitment to GS:SFHS, were available from pre-eclampsia cases and controls which had been identified in chapter 3. Samples were available from 329 women with a history of pre-eclampsia and 658 normotensive controls matched for year of birth, BMI, systolic blood pressure and smoking status. Biomarker studies were performed in a subset of these women (55 cases and 110 matched controls) as described in chapter 6.

2.4.1 Generation Scotland ethical approval and other approvals

Ethical approval had been granted for the GS:SFHS (REC reference number 05/S1401/89) and women who had taken part in GS:SFHS had agreed to be contacted to participate in future studies and had given consent at time of recruitment for the use of their blood and urine samples in other related studies. Access to Generation Scotland data and samples was applied for and granted by the Generation Scotland access committee (reference number GS11088).

For the record linkage study, additional approval was required. The anonymity of study subjects was maintained as outlined in a Privacy Advisory Committee (PAC) application which was filed by the COPS study team and had been approved (Appendix 13).

2.5 The COPS vascular study

2.5.1 Recruitment

The COPS vascular studies took place at the British Heart Foundation Glasgow Cardiovascular Research Centre (BHF GCRC) and recruited 86 women with a history of pre-eclampsia and 80 controls. They were recruited from multiple sources: GS:SFHS, the Proteomics in Pre-eclampsia (PIP) study cohort, blood pressure clinics and some friends and colleagues of participants who contacted us because they had heard about the study and were interested in participating. These women were recruited to the blood pressure group as this group best reflected their age.

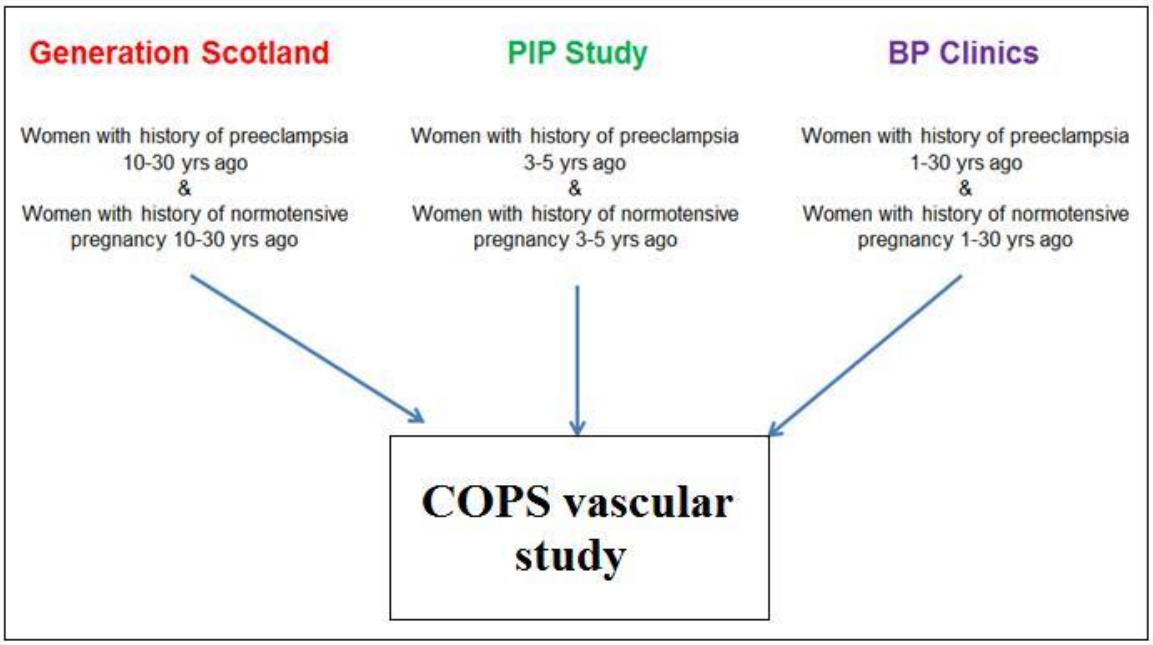


Figure 2.1 COPS vascular study recruitment groups.

2.5.2 Ethical approvals relating to the COPS vascular study

The COPS vascular study was approved as part of the overall COPS study as mentioned in section 2.3. All participants who had previously been recruited to GS:SFHS and PIP had given written informed consent at the time of those studies. GS:SFHS approval is described in section 2.4.1 and PIP REC approval reference 07/S0709/79.
New recruitment of women to the COPS vascular study was carried out according to the Declaration of Helsinki and written informed consent was given (forms in Appendices 1-13)

2.5.3 Study protocol

Inclusion criteria were women who had a pregnancy 1-30 years ago and women were excluded if they were >60 years old, already had established cardiovascular disease or if they were unable to give informed consent. There were deviations from the study protocol only on age of recruitment of women for the purposes of age-matching and these were discussed with R&D and appropriate file note documented in the site file as advised.

2.5.4 **Study visit**

All clinical vascular studies were carried out at the BHF GCRC. Study participants were asked to refrain from smoking or caffeine intake for 6 hours before the appointment and wore loose fitting clothing. Time of last meal, time of last cigarette and time of study visit were all noted. All studies were carried out in quiet dedicated temperature-controlled rooms between 21-23^{o}C.

2.5.5 **Study questionnaire**

The study questionnaire consisted of obstetric history, past medical history, drug history, smoking history and family history (Appendix 10).

2.5.6 **Anthropometric measurements**

Height was measured to the nearest millimetre with shoes removed. Weight was measured using routinely calibrated Seca weighing scales. The same equipment was used for all study visits. Body mass index (BMI) kg/m^2 was calculated as: weight (kg)/height (m)^2.

2.5.7 **Blood pressure**

Participants rested in a sitting position for 5 minutes. The Omron 705-IT device (Omron Corporation, Shimogyo-ku, Kyoto, Japan) was used to take three readings and the average of the 2nd and 3rd blood pressure readings was used for subsequent analysis. Any significant abnormalities were reported to the subject's general practitioner.

2.5.8 **Blood and urine samples**

Blood samples were taken from the antecubital fossa using a standard tourniquet and Vacutainer system. One of the gold topped serum separator (SST) tubes was delivered to NHS Greater Glasgow and Clyde, Western Infirmary biochemistry laboratory for lipid studies. Any significant abnormalities were reported to the patient and the patient's general practitioner. The other samples were placed on ice and centrifuged at 2500 rpm in the laboratory at 4^{o}C for 15 minutes. They were then frozen at -80^{o}C as indicated in Figure 2.2. A mid-stream specimen of urine was collected in a universal container and aliquoted

in the laboratory into 3 separate 1ml aliquots. These were frozen at -80^{o}C. Unique study IDs were given to samples from each participant, starting with the letter "C" for the "COPS" study, then a 5 digit space for sequential numbering beginning at number "00001" for the first patient, and a final letter indicating which recruitment cohort the participant came from e.g "G" for Generation Scotland, "P" for the PIP study and "C" for the blood pressure clinic group. The first patient was given code "C-00001-P" indicating they were from the PIP cohort.

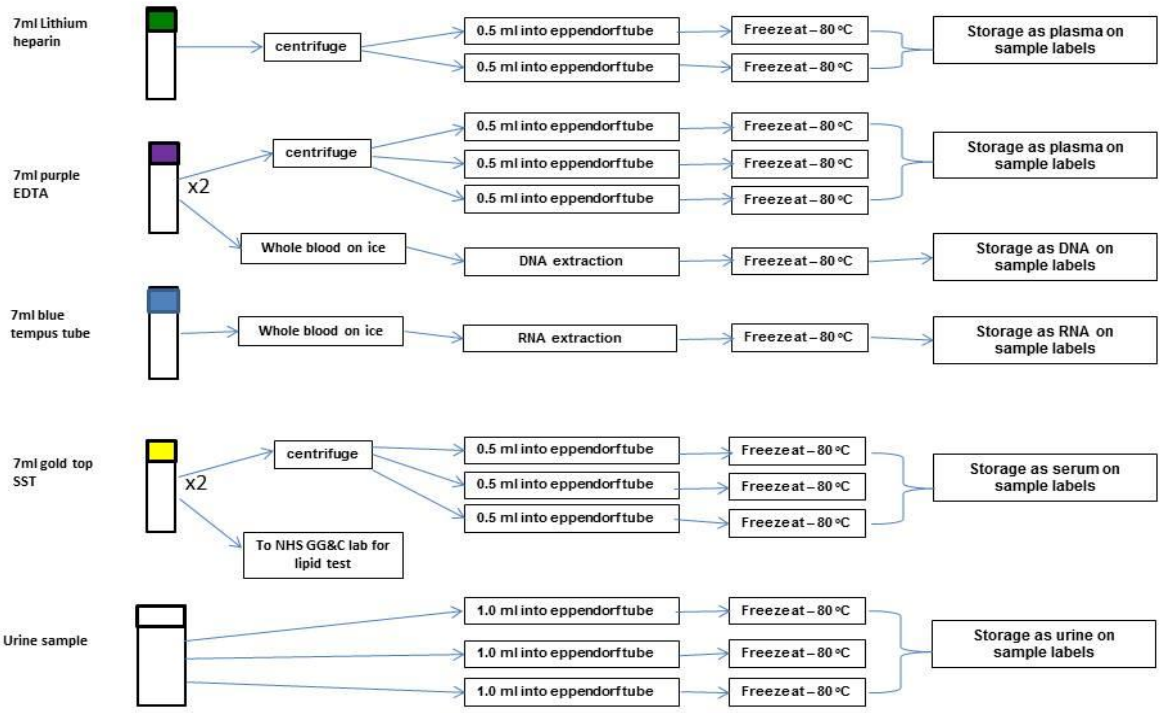


Figure 2.2 Blood and urine sample processing for the COPS vascular study.
6 blood samples were taken as follows: 1x lithium heparin, 2x EDTA, 1x tempus RNA tube, 2x gold SST. EDTA= ethylenediamine tetraacetic acid, SST=serum separator tube, DNA= deoxyribonucleic acid, RNA= ribonucleic acid

2.5.9 **Electrocardiogram**

A standard 12-lead resting electrocardiogram (ECG) was carried out in the supine position. Any significant abnormalities were reported to the participant's general practitioner. It was important to perform ECG prior to commencement of pulse wave analysis and pulse wave velocity as these tests cannot be performed in patients who are not in sinus rhythm.

2.6 **Statistical analysis**

Statistical analyses were performed using Minitab v17 (Minitab Inc, State College, PA, USA) and SPSS v22 (IBM Corp, Armonk, New York, USA). The Kolmogorov-Smirnov test was used to assess normality of data distribution and histograms and plots were manually examined. Independent samples t-test was used for comparison of continuous variables unless data were matched, in which case paired samples t-test was used. If data were not normally distributed, they were transformed. Any continuous variables which remained non-parametric were analysed using the Mann-Whitney U test for comparison of independent samples and Wilcoxon signed rank test if paired. All variables are described as mean ± standard deviation or median (interquartile range) as appropriate unless otherwise stated. Chi-squared test and Fisher's exact test were used to compare categorical variables. Pearson's or Spearman's correlation coefficient were used as appropriate and multiple linear regression to investigate relationships between variables. A p-value of <0.05 was considered significant.

This is a general description of statistics, and any specific methods will be described in the relevant results chapters.

3 The Cardiovascular Consequences of Pre-eclampsia Record Linkage Study

3.1 Introduction

Women with a history of pre-eclampsia are known to be at an increased risk of cardiovascular disease later in life. One of the first descriptions of this was by Adams and MacGillivray et al over 50 years ago (80). Over the past decades, supportive evidence has been compiled to such an extent that, in the American Heart Association's latest guidelines regarding cardiovascular risk in women, pre-eclampsia has been recognised as a risk factor for cardiovascular disease and stroke (89;90).

Many epidemiological studies have addressed risk of death from cardiovascular disease following hypertensive pregnancy disorders (6;81;101;361-363). Risk of cardiovascular diseases in general such as hypertension, cerebrovascular disease and ischaemic heart disease, following hypertensive disorders of pregnancy (6;80;81;83;85;95;97-101;364) have also been addressed.

The effects of parity on future risk of cardiovascular disease must also be considered. There has been conflicting evidence in the literature regarding the influence of parity, especially nulliparity on future cardiovascular disease (365-371). A recent meta-analysis of cardiovascular disease mortality and parity by Lv et al (372) reported a borderline reduction in cardiovascular mortality in ever parous women vs nulliparous. It argued that higher fertility could reflect healthier women at baseline, who would therefore be at a lower risk of cardiovascular mortality. In contrast, one of the most recent studies in this field (370) in the pan-European (EPIC)-CVD study, a case-cohort nested study from the EPIC prospective study, found that in 12,319 women with data on parity and incident coronary heart disease, hazard ratio in parous vs nulliparous women was 1.27 (95% CI 1.09-1.47) and after adjusting for potential confounders this was attenuated to 1.19 (95% CI 1.01-1.41). Hazard ratio appeared to increase as parity increased up to 5 or more children. Childbearing women who had ever breastfed were at a lower risk of coronary heart disease than those who had never breastfed.

Preterm birth in women with pre-eclampsia is also an important risk factor for later cardiovascular event and mortality as evidenced by a large register-based Norwegian study

of incident coronary heart disease after pre-eclampsia (373). Women who gave birth between 1980-2002 were followed for incident major coronary event and mortality. When women with pre-eclampsia were compared to those without pre-eclampsia, the hazard ratio for major coronary event was 2.1 (95% CI 1.73-2.65) for pre-eclampsia, 3.3 (2.37-4.57) for pre-eclampsia combined with babies small for gestational age, and 5.4 (3.74-7.74) for pre-eclampsia in combination with preterm delivery (373).

The study described in this chapter sought to investigate the effect of pre-eclampsia on future cardiovascular event risk in women who had been enrolled in the Generation Scotland Scottish Family Health Study (GS:SFHS) as a representative sample of the Scottish population, by record linkage using data held by the Information Services Division (ISD). Its aims were:

1) to examine the relationship between nulliparous and parous women and cardiovascular disease.
2) to investigate whether women with a history of pre-eclampsia were at an increased risk of cardiovascular disease later in life.
3) to perform survival analysis to further evaluate the relationship between cardiovascular events and pre-eclampsia.

3.2 Methods

3.2.1 Ethical approval

This study was approved by the West of Scotland Research Ethics Committee (reference 12/WS/0306). Approval for data linkage was obtained from the Privacy Advisory Committee (PAC) of NHS National Services Scotland. The study adhered to the principles of the Declaration of Helsinki and all participants had provided written informed consent for access to their health records. Ethical approval for the Generation Scotland study itself is outlined in Chapter 2.

3.2.2 Generation Scotland and Record Linkage

The Generation Scotland: Scottish Family Health Study (GS:SFHS) was used. This resource has a large amount of data available from questionnaires and clinical

examinations. Blood and urine samples were also taken, processed and stored at the time of recruitment. More than 20,000 participants have been recruited to this study from the Scottish population (359;360). Recruitment between 2006 and 2011 was based in Aberdeen, Dundee, Glasgow and Edinburgh.

Record linkage (as outlined in Figure 3.1) was performed to enable cardiovascular outcomes to be linked with the study participant and also obstetric history (which was not part of the GS:SFHS study questionnaire). Requirements for anonymity of study subjects (as agreed with the Privacy Advisory Committee) was maintained and facilitated by the GS:SFHS team in Edinburgh (Archie Campbell) working with the Information Services Division (ISD NHS Scotland) and Health Informatics Centre (HIC). A new identification number was allocated for each participant, which for the purposes of this study was labelled the 'ISD' number (number from the Information Services Division). For the purposes of data analysis, through the ISD number it was possible to link the SMR01 and SMR02 data, which was sent via the secure NHS server. A description of the process used to link SMR01 and SMR02 data is found in the Statistical analysis section 3.2.4.

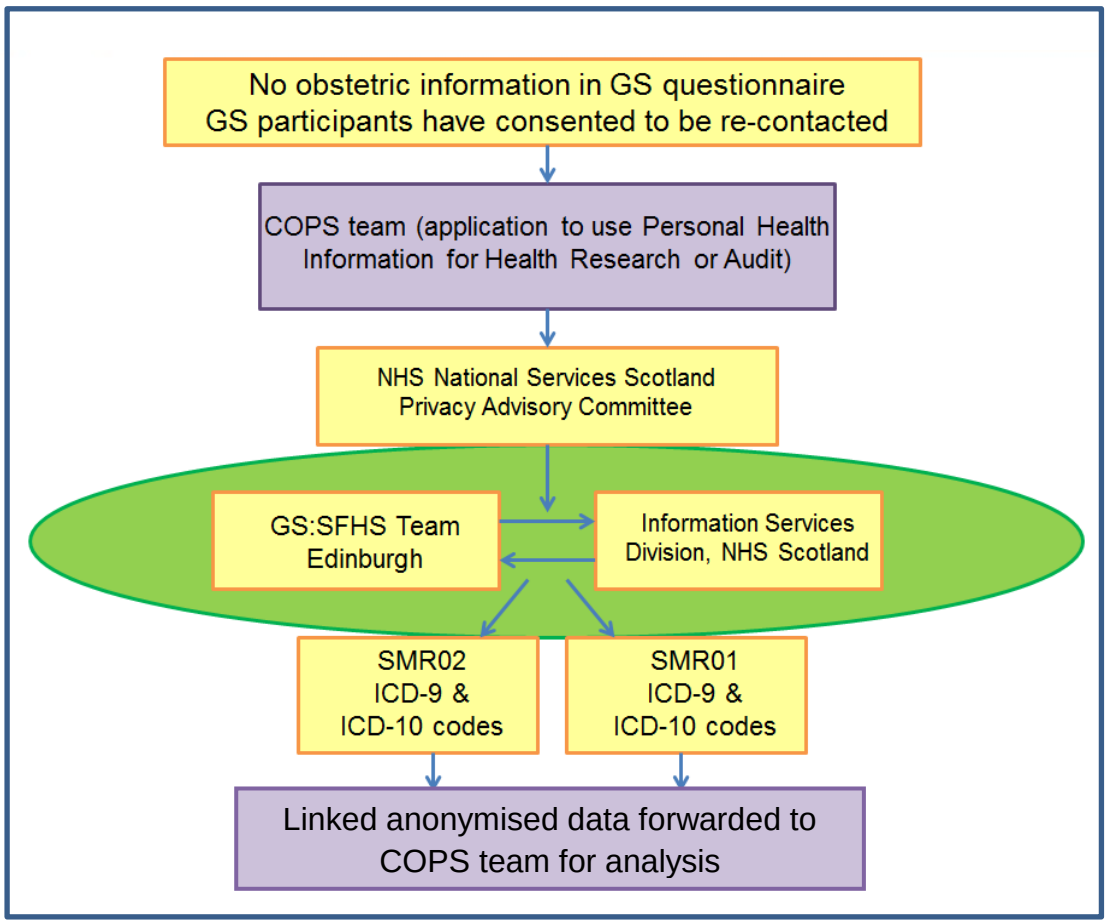


Figure 3.1 Outline of application process and acquisition of data for record linkage study.

The Information Services Division have assessed the quality of SMR01 and SMR02 data (374;375) and data collection has been >99% complete since the ICD-9 classifications would have started at the end of the 1970s (376).

Once the relevant SMR01 and SMR02 data had been collated by ISD it was forwarded in a format which facilitated merging of datasets, with a unique ISD number linking the information between SMR01 and SMR02. The data remained fully anonymised without possibility of access to CHI numbers or other patient personal identifiers. The data obtained was stored on secure servers at the University of Glasgow.

3.2.3 **International Classification of Disease (ICD)**

The International Classification of Disease (ICD) is a coding system which was developed to promote uniformity of disease diagnoses in published literature by the World Health Organization that provides an internationally recognised method of coding diseases in order to categorise mortality and morbidity statistics. The tenth and most recent revision (ICD-10) was introduced in 1999. Revisions are currently underway for new ICD-11 classifications and a final version for approval at the World Health Organisation World Health Assembly is expected in 2018. For the purposes of this study ICD-9 classifications were also used as these were introduced in 1979-1998. I excluded pregnancies classified as pre-eclampsia by earlier ICD-8 classification as these diagnoses were judged to be less reliable considering the changing diagnostic criteria for hypertensive disorders of pregnancy over the past 40years.

3.2.3.1 ***Hospital admission SMR01 data***

Cardiovascular events were only included if they were listed as the main condition for the hospital admission. SMR01 data were coded as follows: Rheumatic heart diseases (ICD-9 390-398, ICD-10 I00-09), hypertensive diseases (ICD-9 401-405, ICD-10 I10-15), ischaemic heart diseases (ICD-9 410-414, ICD-10 I20-I25), pulmonary heart diseases (ICD-9 415-417, ICD-10 I26-28), other forms of heart disease (ICD-9 420-429, ICD-10 I30-I52), cerebrovascular diseases (ICD-9 430-438, ICD-10 I60-69), diseases of the arteries, arterioles and capillaries (ICD-9 440-448, ICD-10 I70-79) and other circulatory diseases (ICD-9 459, ICD-10 I95-99) . Any disorders of the venous system e.g. ICD-9 codes 451-458 and ICD-10 codes I80-89 were excluded. The full breakdown of number of entries for each code is given in Table 3.9.

3.2.3.2 ***Maternity SMR02 data***

As previously mentioned, for the purposes of this study pregnancies dating as far back as 1980 were included. Regarding SMR02 data, women to be classified as a ‘case’ of pre-eclampsia had ICD-9 codes 642-4, 642-5, 642-6 and 642-7 or ICD-10 codes O11, O14-0, O14-1, O14-2, O14-9, O15-0, O15-1, O15-2 and O15-9. Women with hypertensive disorders of pregnancy but not pre-eclampsia were excluded and had ICD-9 codes 642-0,

642-1, 642-2, 642-3, 642-9, 646-2, 648-0, 648-8 and ICD-10 codes O10, O12, O13, O16, O24. A full description of the individual codes is given in Tables 3.1 and 3.2.

3.2.3.3 *Generation Scotland data*

Information available from the Generation Scotland recruitment visit as analysed in this chapter was made up of several different components: The age at time of visit, anthropometric measurements, clinical measurements (blood pressure and heart rate), biochemistry and self-reporting of clinical diagnoses (hypertension and diabetes) and smoking history.

3.2.4 Statistical analysis

Initial preparation of the dataset using R (http://R-project.org) was performed by Mr Scott Robinson. This consisted of identification and removal of duplicate records, merging of SMR01 and SMR02 tables, homogenisation of ICD codes with the use of regular expressions and calculation of data used directly for analysis for example survival times and the categorisation of participants. Following this, biochemistry and other phenotype data were then merged with this table using a custom visual basic script run in Microsoft Excel.

Statistical analysis was then performed by the author using IBM SPSS Statistics for Windows (Version 22.0, Armonk, NY: IBM Corp, 2013). For comparison of continuous variables independent samples t-test or Mann-Whitney U test were used depending on the distribution of the data. Categorical variables were compared using Chi-squared test. Continuous variables were summarised using mean ± standard deviation or medians and inter-quartile range, depending on data distribution. Birth weight was adjusted for gestational age using z-scores (standard deviation scores). Z-score calculations were performed using the 2003 Fenton growth chart (377) This particular chart was used in preference to more modern gender-specific growth charts because information on gender of baby was not available to us in the extracted SMR02 data. However, the 2003 Fenton chart is based on data from population studies with large sample sizes. Time–to-event analysis was performed using Kaplan-Meier survival curves and Cox proportional hazards model was used to evaluate the hazard ratio and its confidence intervals.

3.3 Results

3.3.1 Identification of women with a history of pre-eclampsia in the GS:SFHS

The index pregnancy was defined as the first pregnancy in normotensive women and the first pre-eclamptic pregnancy in those with pre-eclampsia (according to ICD-9 and ICD-10 classification). See Tables 3.1 and 3.2 for inclusion and exclusion classifications for pre-eclampsia; also included is the number of times each code was entered for SMR02 data. None of the pregnancies had gestations of <20weeks; if they had been present they would have been excluded.

The flow diagram in Figure 3.2 demonstrates the identification of pre-eclampsia cases and normotensive controls within the Generation Scotland cohort. There were 9,732 women with SMR01 and SMR02 data available. Of them 3,693 were never pregnant and 6,039 women had 11,170 pregnancies. Thirteen women experienced a cardiovascular event before their first pregnancy and were therefore excluded, as cardiovascular event was the endpoint of interest. This left a total of 6,026 women with 11,146 pregnancies. A further 496 women (with 1,004 pregnancies) were excluded as they had experienced other hypertensive disorders of pregnancy. Of the remaining 5,530 women with 10,142 pregnancies, another 277 women (with 559 pregnancies) were excluded because they had less reliable ICD-8 coding for pre-eclampsia and no ICD-9 or ICD-10 code. Their classification as a "case" would not necessarily have been correct, neither could they reasonably be used as a "control". A total of 5,253 women with 9,583 pregnancies remained. Of these, 4,922 women had 8,880 normotensive pregnancies and 331 women with 703 pregnancies were in the pre-eclampsia group. Out of the 331 women, there were 359 actual pre-eclamptic pregnancies. Pre-eclampsia occurred in the first pregnancy in 237/331 (71.6%) women, the second pregnancy in 76/331 (23%) women and the third pregnancy in 18/331 (5.4%) women. A total of 303/331 (91.5%) women experienced pre-eclampsia in only one pregnancy, and 28/331 (8.5%) experienced pre-eclampsia in two pregnancies.

Figure 3.2 Identification of women with pre-eclampsia in GS:SFHS

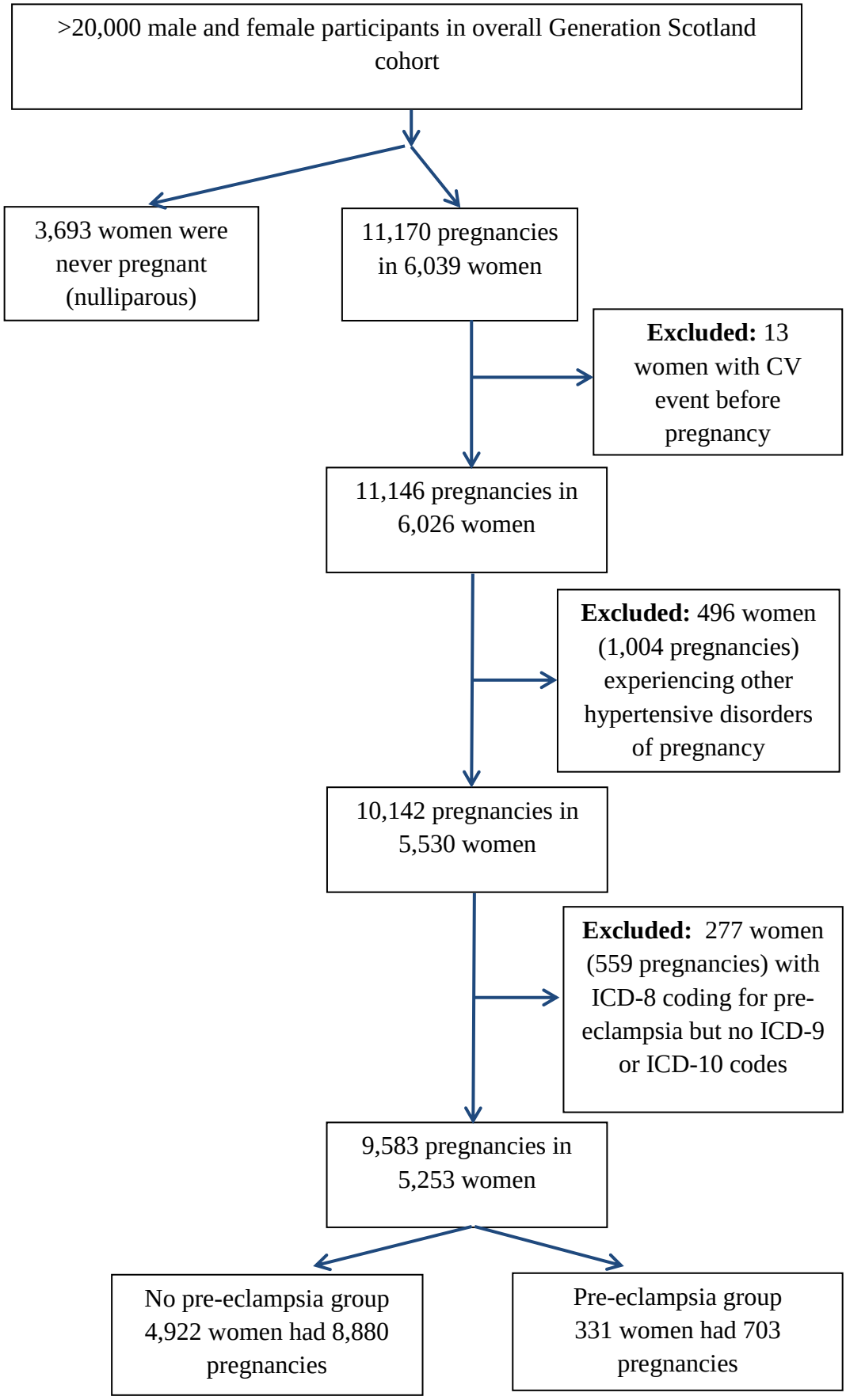


Table 3.1 Breakdown of pre-eclampsia ICD codes in 331 women for inclusion

ICD-9 codes	**Description**	**Number of PE entries in SMR02 data**
642-4	Mild or unspecified pre-eclampsia	259
642-5	Severe pre-eclampsia	45
642-6	Eclampsia	4
642-7	Pre-eclampsia or eclampsia superimposed on pre-existing hypertension	1
ICD-10 codes		
O11	Pre-existing hypertension with superimposed proteinuria	1
O14-0	Mild to moderate pre-eclampsia	8
O14-1	Severe pre-eclampsia	7
O14-2	HELLP syndrome	0
O14-9	Unspecified pre-eclampsia	39
O15-0	Eclampsia in pregnancy	1
O15-1	Eclampsia in labour	0
O15-2	Eclampsia in the puerperium	1
O15-9	Eclampsia unspecified as to time period	0
	TOTAL number of PE codes for 331 women with 703 pregnancies	366[†]

PE = pre-eclampsia, HELLP = haemolysis, elevated liver enzymes, low platelets, ICD = International Classification of Diseases, SMR= Scottish Morbidity Records.
[†] N.B. Some pre-eclamptic pregnancies were given 2 different "pre-eclampsia category" codes for one admission. This data reflects the fact that there were 366 pre-eclampsia codes entered in total for 331 women during the course of their 703 pregnancies.

Table 3.2 Breakdown of ICD codes in 496 women with other hypertensive disorders of pregnancy, excluded from further analysis

ICD-9 codes	Description	Number of entries in SMR02 data
642-0	Benign essential hypertension	5
642-1	Hypertension secondary to renal disease	1
642-2	Other pre-existing hypertension	1
642-3	Transient hypertension	16
642-9	Unspecified hypertension	326
646-2	Unspecified renal disease without hypertension	14
648-0	Diabetes mellitus	7
648-8	Abnormal glucose tolerance	22
ICD-10 codes		
O-10	Pre-existing hypertension	15
O-12	Gestational oedema and proteinuria without hypertension	35
O-13	Gestational hypertension without proteinuria	82
O-16	Unspecified maternal hypertension	32
O-24	Diabetes mellitus in pregnancy	22
	TOTAL number of coding entries for the 496 women (1,004 pregnancies) excluded	578*

PE = pre-eclampsia, ICD = International Classification of Diseases, SMR= Scottish Morbidity Records.

*** N.B. Some pregnancies were different codes in the table during a single pregnancy. This table reflects the number of times each individual code was used for the 1,004 pregnancies in 496 women.**

3.3.2 Descriptive statistics for Generation Scotland data

3.3.2.1 *Descriptive statistics for nulliparous women vs pregnant women*

It is important to clarify that for the purposes of describing the work presented in this chapter, the word “nulliparous” has been used to describe women who have not given birth. Some studies describe pregnant women as “nulliparous” if they are experiencing their first pregnancy, i.e. they have never been parous before but are currently pregnant. This thesis describes such women as “primigravida” (pregnant for the first time) and “primiparity” as having given birth to their first child. The word “parous” is used to mean women who have given birth.

The following table shows the differences between baseline characteristics of nulliparous and parous women at time of recruitment to the Generation Scotland study. Of note is the fact that nulliparous women had a significantly higher body mass index (BMI), systolic blood pressure (SBP) and diastolic blood pressure (DBP). There were also significant differences in most biochemical parameters, with a higher total cholesterol in women who had given birth. On Chi-squared test there was a significant difference in current diagnoses of hypertension and diabetes between the two groups, with women who had never been pregnant having a higher proportion of cases of hypertension and diabetes. There was also a significant difference in smoking between the two groups with parous women being more likely to smoke (Table 3.3).

Table 3.3 Comparison of clinical and biochemical measurements taken at time of recruitment to the Generation Scotland study in the remaining 8,946 women following exclusions

Measurement	Nulliparous* (N=3,693)	Pregnant ever* (N=5,253)	p-value†	Age-adjusted p-value
Age (yrs)	49.4 ± 18.1	46.5 ± 10.9	**<0.001**	**N/A**
Height (cm)	161.9 ± 6.8	162.6 ± 6.3	**<0.001**	**0.032**
Weight (kg)	70.2 ± 15.4	69.7 ± 14.7	0.182	0.139
BMI (kg/m^2)	26.8 ± 5.8	26.3 ± 5.4	**0.001**	**0.015**
Mean SBP (mmHg)	130.9 ± 19.3	125.6 ± 17.1	**<0.001**	**<0.001**
Mean DBP (mmHg)	78.5 ± 10.1	77.8 ± 10.0	**0.004**	0.608
Mean HR (bpm)	71.0 ± 11.1	70.4 ± 10.7	**0.009**	**0.001**
Glucose (mmol/L)	4.8 ± 1.0	4.7 ± 1.0	**<0.001**	**0.007**
Total cholesterol (mmol/L)	5.1 ± 1.1	5.2 ± 1.1	**<0.001**	**<0.001**
HDL cholesterol (mmol/L)	1.583 ± 0.414	1.582 ± 0.410	0.890	0.175
Sodium (mmol/L)	139.6 ± 2.5	139.8 ± 2.3	**0.003**	**<0.001**
Potassium (mmol/L)	4.17 ± 0.46	4.13 ± 0.41	**<0.001**	**<0.001**
Urea (mmol/L)	5.0 ± 1.6	4.8 ± 1.2	**<0.001**	**<0.001**
Creatinine (μmol/L)	67.9 ± 12.3	66.4 ± 10.4	**<0.001**	**<0.001**
Current Hypertension	606/3637 (16.7%)	492/5143 (9.6%)	**<0.001**	
Current Diabetes	123/3637 (3.4%)	72/5143 (1.4%)	**<0.001**	
Current smoker	563/3583 (15.7%)	933/5100 (18.3%)	**0.002**	

SBP = systolic blood pressure, DBP = diastolic blood pressure, HR = heart rate, HDL = high density lipoprotein, BMI = body mass index

*** In order to demonstrate the direction of difference, data are displayed as mean ± standard deviation. Proportions are given for categorical data, with the denominator reflecting the number of data entries available for that parameter.**

† p-value is for independent samples t-test if continuous data normally distributed, and Mann-Whitney U test if continuous data not normally distributed. Chi-squared test results are given for categorical data.

3.3.2.2 *Descriptive statistics for women with pre-eclampsia vs normotensive pregnancies*

Women with a history of pre-eclampsia had significantly higher weight and BMI at time of recruitment to the Generation Scotland study than women with normotensive pregnancies. They also had significantly higher BMI, SBP, DBP and heart rate and significant differences in current diagnosis of hypertension and diabetes, with a greater proportion of women having these conditions in the pre-eclampsia group (Table 3.4).

Comparison of birth characteristics of the index pregnancies (as used for survival analysis, described further in section 3.3.4) revealed that there was no difference in age at index pregnancy and women with pre-eclampsia gave birth to lower birth weight babies and gave birth at an earlier gestation. However, on adjusting birth weight for gestational age using z-scores, there was no statistically significant difference between groups. Chi-squared test revealed that women with a history of pre-eclampsia were more likely to have had a C-section at delivery, and to have had a multiple gestation pregnancy (Table 3.5). For twin pregnancies, the mean of two birth weights was used in statistical analysis.

Table 3.4 Comparison of clinical and biochemical measurements taken at time of recruitment to the Generation Scotland study in 5,253 women with pregnancies

Measurement	Normotensive pregnancy* (N=4,922)	Pre-eclampsia* (N=331)	p-value†	Age-adjusted p-value
Age (yrs)	46.4 ± 11.0	48.3 ± 8.5	**0.006**	**N/A**
Height (cm)	162.7 ± 6.3	162.0 ± 6.0	0.066	0.192
Weight (kg)	69.4 ± 14.5	73.2 ± 16.8	**<0.001**	**<0.001**
BMI (kg/m^2)	26.2 ± 5.3	27.9 ± 6.2	**<0.001**	**<0.001**
Mean SBP (mmHg)	125.1 ±17.0	132.7 ± 16.5	**<0.001**	**<0.001**
Mean DBP (mmHg)	77.5 ± 9.9	83.0 ± 9.3	**<0.001**	**<0.001**
Mean HR (bpm)	70.3 ± 10.7	72.4 ± 11.1	**0.001**	**<0.001**
Glucose (mmol/L)	4.7 ± 1.0	4.8 ± 1.1	**0.002**	**0.017**
Total cholesterol (mmol/L)	5.2 ± 1.1	5.3 ± 1.1	0.503	0.525
HDL cholesterol (mmol/L)	1.58 ± 0.41	1.57 ± 0.38	0.587	0.188
Sodium (mmol/L)	139.8 ± 2.3	140.0 ± 2.2	0.202	0.282
Potassium (mmol/L)	4.13 ± 0.41	4.11 ± 0.38	0.619	0.332
Urea (mmol/L)	4.8 ± 1.2	4.9 (1.3)	0.137	0.503
Creatinine (μmol/L)	66.3 ± 10.4	67.4 ± 10.4	**0.05**	0.095
Current Hypertension	398/4818 (8.3%)	94/325 (28.9%)	**<0.001**	
Current Diabetes	59/4818 (1.2%)	13/325 (4.0%)	**<0.001**	
Current smoker	886/4777 (18.5%)	47/323 (14.6%)	0.072	

SBP = systolic blood pressure, DBP = diastolic blood pressure, HR = heart rate, HDL = high density lipoprotein, BMI = body mass index. * Data are displayed as mean ± standard deviation to demonstrate the direction of difference. Proportions are given for categorical data, with the denominator reflecting the number of data entries available for that parameter.

† p-value is for independent samples t-test if continuous data normally distributed, and Mann-Whitney U test if continuous data not normally distributed. Chi-squared test results are given for categorical data.

Table 3.5 Comparison of birth data in women with pre-eclampsia vs women with normotensive pregnancy

Measurement	**Normotensive pregnancy (N=4,922)**	**Pre-eclampsia (N=331)**	**p-value**
Age at index pregnancy (yrs)*	27 ± 5	27 ± 5	0.389
Birth weight at index pregnancy (g)*	3384 ± 548	3216 ± 699	**<0.001**
Gestation at index pregnancy birth (weeks)*	39.6 ± 1.9	38.7 ± 2.4	**<0.001**
Z-score for birth weight	-0.1612 ± 0.86	-0.1861 ± 0.89	0.456
Number of multiple gestation pregnancies†	66/4922 (1.3%)	11/331 (3.3%)	**0.004**
C-section required at delivery†	791/4922 (16.1%)	92/331 (27.8%)	**<0.001**

*** Data described as mean ± standard deviation for the purposes of this table. Continuous data were compared using parametric or non-parametric methods depending on distribution.**

†Chi-test was used to compare data for categorical outcomes.

Table 3.6 Table of descriptive results for pre-eclampsia cases and normotensive controls in 5,253 women by cardiovascular events vs no cardiovascular events

	Cardiovascular event (N=218)			**No cardiovascular event (N=5,035)**		
Measurement	**Normotensive pregnancy (N=193)**	**Pre-eclampsia (N=25)**	**p-value**	**Normotensive pregnancy (N=4,729)**	**Pre-eclampsia (N=306)**	**p-value**
Age (yrs)	54 (10)	53 (9)	0.578	47 (17)	49 (12)	**0.010**
Height (cm)	161.2 ± 6.0	160.0 ± 6.5	0.326	163.0 (8.2)	162.0 (9.9)	0.135
Weight (kg)	70.7 (23.5)	76.4 (26.0)	0.858	66.7 (17.1)	69.3 (19.7)	**<0.001**
BMI (kg/m^2)	27.2 (9.7)	27.0 (10.5)	0.645	25.2 (6.4)	26.6 (7.1)	**<0.001**
Mean SBP (mmHg)	132 (30)	135 (22)	0.445	122 (21)	130 (23)	**<0.001**
Mean DBP (mmHg)	79 (15)	81 (12)	0.283	77 (12)	82 (12)	**<0.001**
Mean HR (bpm)	70 (12)	73 (11)	0.225	69 (14)	72 (14)	**<0.001**
Glucose (mmol/L)	4.7 (0.7)	4.8 (1.8)	0.243	4.6 (0.5)	4.6 (0.7)	**0.007**
Total cholesterol (mmol/L)	5.2 ± 1.2	5.1 ± 1.1	0.694	5.1 (1.4)	5.2 (1.3)	0.425
HDL cholesterol (mmol/L)	1.5 ± 0.4	1.5 ± 0.4	0.675	1.5 (0.5)	1.5 (0.5)	0.564
Sodium (mmol/L)	140 (4)	140 (2)	0.317	140 (3)	140 (2)	0.109
Potassium (mmol/L)	4.1 (0.5)	4.1 (0.6)	0.465	4.1 (0.4)	4.1 (0.4)	0.692
Urea (mmol/L)	5.1 (1.7)	5.1 (1.2)	0.932	4.6 (1.6)	4.8 (1.5)	0.204
Creatinine (μmol/L)	65 (12)	73 (23)	**0.018**	65 (13)	66 (12)	0.188
Current Hypertension	47/187 (25.1%)	13/25 (52%)	**0.005**	351/4631 (7.6%)	81/300 (27%)	**<0.001**
Current Diabetes	10/187 (5.3%)	5/25 (20%)	**0.007**	49/4631 (1.1%)	8/300 (2.7%)	**0.012**
Current smoker	46/186 (24.7%)	7/25 (28%)	0.723	840/4591 (18.3%)	40/298 (13.4%)	**0.034**

BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, HR = heart rate, HDL = high density lipoprotein.
† p-value is for independent samples t-test if continuous data normally distributed, and Mann-Whitney U test if continuous data not normally distributed. Chi-squared test or Fisher's exact test results are given for categorical data.

Table 3.7 Table of descriptive results for cardiovascular events vs no cardiovascular events by pre-eclampsia vs normotensive pregnancy in 5,253 women

	Pre-eclampsia (N=331)			**No pre-eclampsia (N=4,922)**		
Measurement	**No CV event (N=306)**	**CV event (N=25)**	**p-value**	**No CV event (N=4,729)**	**CV event (N=193)**	**p-value**
Age (yrs)	49 (12)	53 (9)	**0.008**	47 (17)	54 (10)	**<0.001**
Height (cm)	162.0 (9.9)	161.0 (8.7)	0.088	163.0 (8.2)	161.0 (8.0)	**0.003**
Weight (kg)	69.3 (19.6)	76.4 (26.0)	0.683	66.7 (17.1)	70.7 (23.5)	**0.001**
BMI (kg/m^2)	26.6 (7.1)	27.0 (10.5)	0.331	25.2 (6.4)	27.2 (9.7)	**<0.001**
Mean SBP (mmHg)	130 (23)	135 (22)	0.449	122 (21)	132 (30)	**<0.001**
Mean DBP (mmHg)	83 ± 9	81 ± 9	0.300	77 (12)	79 (15)	**0.009**
Mean HR (bpm)	73 ± 11	73 ± 11	0.863	69 (14)	70 (17)	0.458
Glucose (mmol/L)	4.6 (0.7)	4.8 (1.8)	0.076	4.6 (0.5)	4.7 (0.7)	**<0.001**
Total cholesterol (mmol/L)	5.3 ± 1.1	5.1 ± 1.1	0.347	5.1 (1.4)	5.1 (1.3)	0.264
HDL cholesterol (mmol/L)	1.5 (0.5)	1.5 (0.4)	0.818	1.5 (0.5)	1.5 (0.6)	**0.015**
Sodium (mmol/L)	140 (2)	140 (2)	0.190	140 (3)	140 (4)	0.347
Potassium (mmol/L)	4.1 (0.4)	4.1 (0.6)	0.922	4.1 (0.4)	4.1 (0.5)	**0.028**
Urea (mmol/L)	4.8 (1.5)	5.1 (1.2)	0.143	4.6 (1.6)	5.1 (1.7)	**<0.001**
Creatinine (μmol/L)	66.8 ± 9.4	75.2 ± 17.0	**0.028**	65 (13)	65 (12)	0.416
Current Hypertension	81/300 (27%)	13/25 (52%)	**0.008**	351/4631 (7.6%)	47/187 (25.1%)	**<0.001**
Current Diabetes	8/300 (2.7%)	5/25 (20%)	**<0.001**	49/4631 (1.1%)	10/187 (5.3%)	**<0.001**
Current smoker	40/298 (13.4%)	7/25 (28%)	**0.047**	840/4591 (18.3%)	46/186 (24.7%)	**0.027**

BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, HR = heart rate, HDL = high density lipoprotein.
† p-value is for independent samples t-test if continuous data normally distributed, and Mann-Whitney U test if continuous data not normally distributed. Chi-squared test or Fisher's exact test results are given for categorical data.

Further analysis of parous women revealed that amongst the 218 women with cardiovascular events (see Table 3.6), there were generally no significant differences in anthropometric or biochemical measurements in those with pre-eclampsia vs those with normotensive pregnancy, except for a significantly higher creatinine in women with pre-eclampsia. On Chi-squared analysis, those with a history of pre-eclampsia had a significantly higher proportion with a current diagnosis of hypertension or diabetes. In women who had not experienced any cardiovascular events (N=5,035) women with pre-eclampsia had a significantly higher BMI, SBP, DBP and heart rate later in life. Chi-squared analysis also revealed a higher proportion of currently hypertensive and diabetic women in the pre-eclampsia group. There were a significantly higher proportion of current smokers in the normotensive group in comparison with the pre-eclampsia group (Table 3.6).

When grouping the 5,253 parous women differently and dividing them up by pre-eclampsia status (pre-eclampsia N=331 vs normotensive N=4,922) it was then possible to analyse differences between those with cardiovascular events vs no cardiovascular events (Table 3.7). In the pre-eclampsia group N=331, there was a significantly higher creatinine in women with a cardiovascular event and women with a cardiovascular event also had a significantly higher proportion of current diagnosis of hypertension and diabetes and significantly more were current smokers in comparison with pre-eclamptic women who had not experienced a cardiovascular event since pregnancy. However, women in the cardiovascular event group were statistically significantly older at time of recruitment to Generation Scotland than those who did not experience a cardiovascular event.

In the group of women who did not experience pre-eclampsia (N=4,922) the age at time of recruitment to Generation Scotland was also statistically significantly different, with women in the cardiovascular event group (N=193) being older at time of recruitment. These women had significantly higher BMI, SBP and DBP than women without any cardiovascular events and they also had a higher glucose. Higher proportions of women in the cardiovascular event group had current diagnosis of hypertension, diabetes and were more likely to be current smokers (Table 3.7), a similar pattern to the pre-eclampsia group described above.

3.3.3 Summary of cardiovascular event data

The number of entries for each individual cardiovascular diagnosis is presented in Table 3.9. A cardiovascular event code denotes the main cardiovascular diagnosis for that specific hospital admission. Some people had more than one hospital admission with a cardiovascular event. In the 333 nulliparous women with cardiovascular events there were 909 separate cardiovascular admissions. Of the 218 parous women with cardiovascular events there were 634 separate hospital admissions, and in the 25 women with cardiovascular event after a pregnancy complicated by pre-eclampsia, there were 57 hospital admissions.

As Table 3.9 shows, there were cardiovascular events in 9% of women who never had a pregnancy, 4.2% of women with pregnancies, and in 7.6% of women with a history of pre-eclampsia in pregnancy. A Chi-squared test confirms a statistically significant difference in cardiovascular events between nulliparous women and women with at least one pregnancy, with test statistic 88.87 and p-value <0.001 (see Table 3.8).

Table 3.8 Comparison of cardiovascular events in nulliparous vs parous women

	Never pregnant	Pregnant
Cardiovascular event ever	333	218
No cardiovascular event ever	3,360	5,035
Total	3,693	5,253

Table 3.9 Summary of SMR01 data: Cardiovascular events described by ICD-9 and ICD-10 category

Description of CV event*	**ICD 9 codes**	**ICD 10 codes**	**Breakdown of CV events in the 333/3,693 (9%) of never pregnant women who had CV events**	**Breakdown of CV events in the 218/5,253 (4.2%) of all pregnant women who had CV events**	**Breakdown of CV events in the 25/331 (7.6%) of women with pre-eclampsia who had CV events**
Rheumatic heart disease	390-398	I00-09	4	4	0
Hypertensive diseases	401-405	I10-15	25	22	8
Ischaemic heart disease	410-414	I20-25	434	195	11
Pulmonary heart diseases	415-417	I26-28	31	32	2
Other forms of heart disease	420-429	I30-52	212	132	21
Cerebrovascular diseases	430-438	I60-69	76	84	10
Diseases of arteries, arterioles and capillaries	440-448	I70-79	116	160	5
Other and unspecified circulatory disorders	459	I95-99	11	5	0
		Total CV events by code	**909**	**634**	**57**

CV = cardiovascular. * This describes the main cardiovascular diagnosis per cardiovascular event entry in the SMR01 data and each time one code was used equates to a cardiovascular admission. The total cardiovascular events in the totals column is therefore the total number of separate cardiovascular admissions to hospital for that column.

Graphical presentation of individual cardiovascular events in the pregnancy group revealed similar patterns for all cardiovascular events and first cardiovascular event (see Figures 3.3-3.6).

Comparison of these pie charts of first cardiovascular event reveals that generally the types of cardiovascular diagnoses were ischaemic heart disease, other forms of heart disease, cerebrovascular disease and diseases of the arteries, arterioles and capillaries. In the 25 women with cardiovascular event after pre-eclampsia, hypertensive diseases also featured in the commonest first cardiovascular events documented.

Chi-squared test (see Table 3.10) revealed a statistically significant difference in cardiovascular events between women with pre-eclampsia and those with a normotensive pregnancy, chi-test statistic 10.283, p-value 0.0013.

Table 3.10 Comparison of cardiovascular events in women with pre-eclampsia vs normotensive pregnancy

	No pre-eclampsia	Pre-eclampsia
Cardiovascular event ever	193	25
No cardiovascular event ever	4,729	306
Total	4,922	331

Figure 3.3 Summary of all 634 cardiovascular events in the 218 women with cardiovascular events after pregnancy

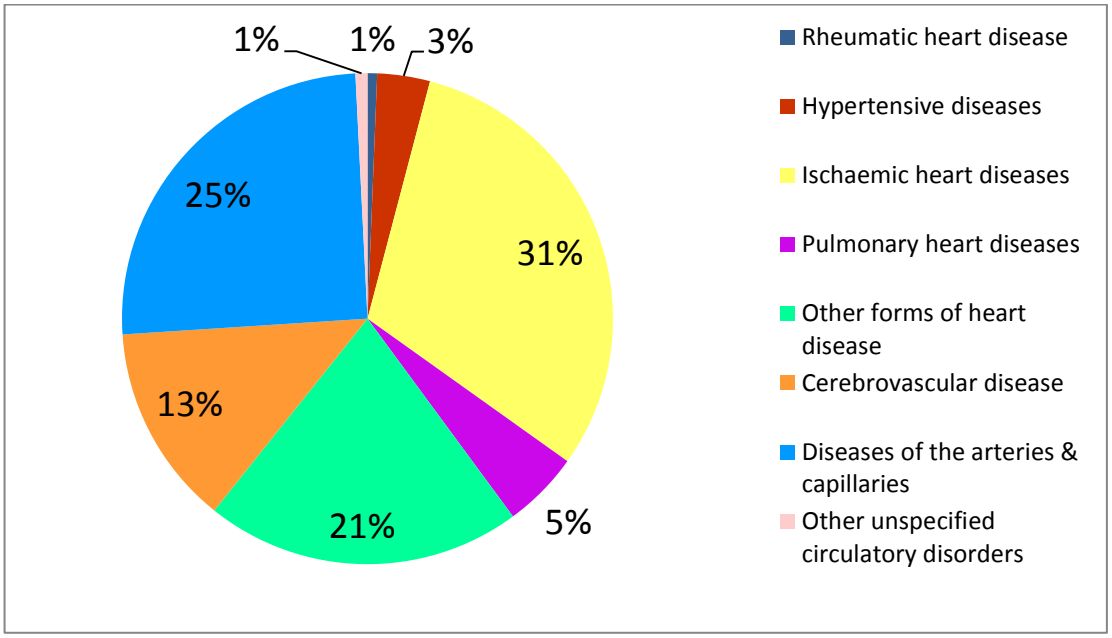


Figure 3.4 Summary of all 57 cardiovascular events in the 25 women with cardiovascular events after pre-eclampsia

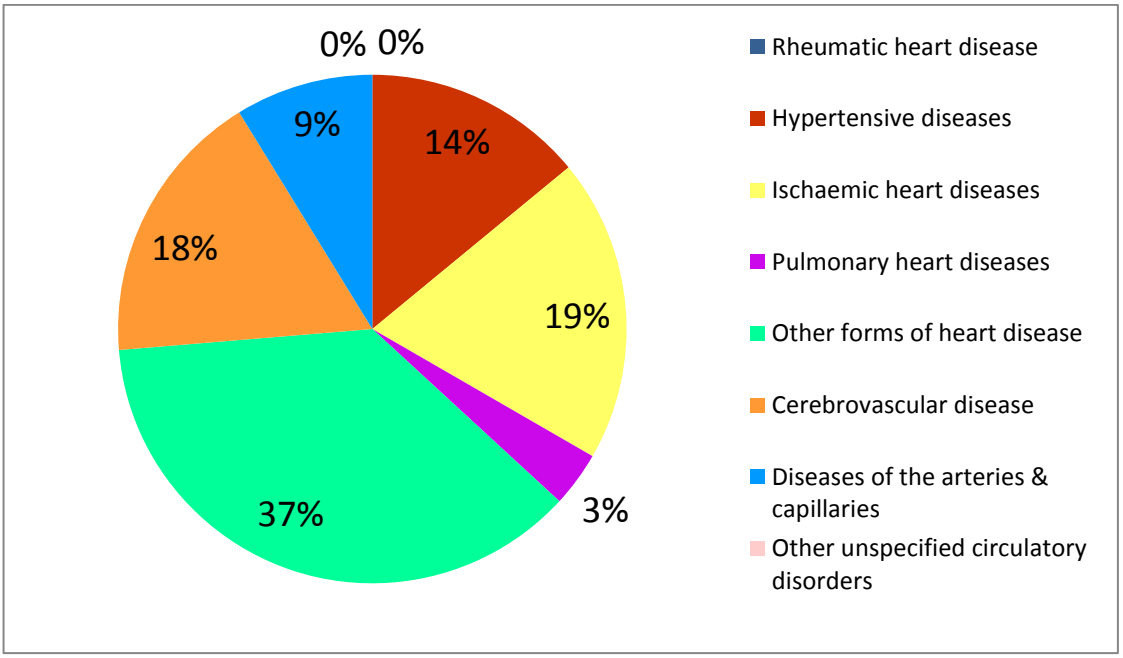


Figure 3.5 First cardiovascular event in the 218 women with cardiovascular events after pregnancy

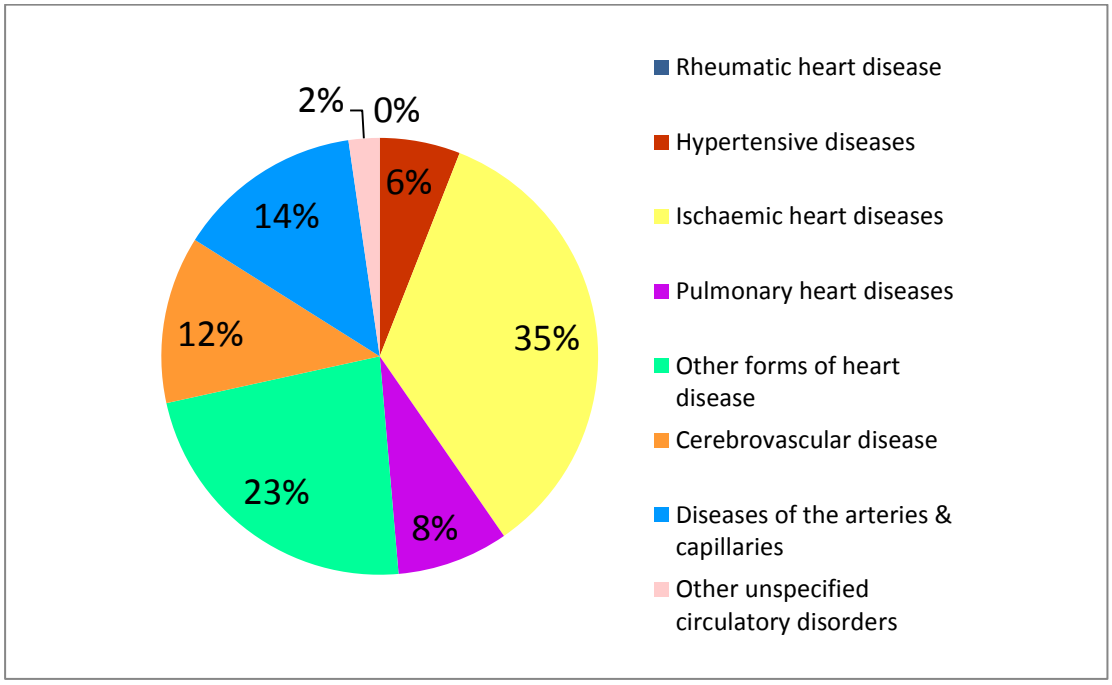
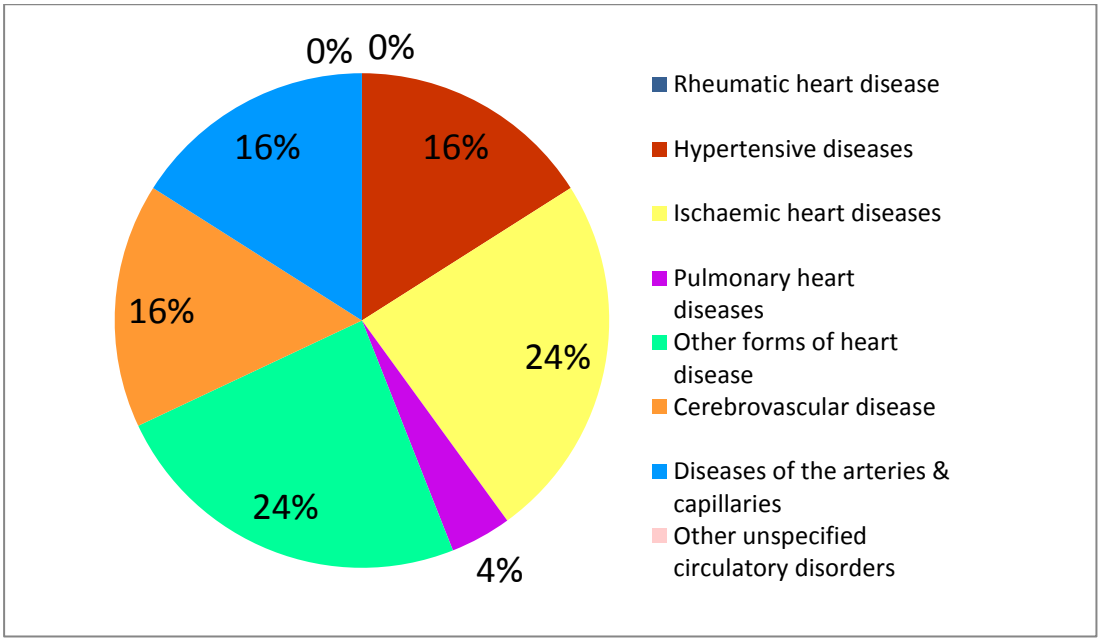


Figure 3.6 First cardiovascular event in the 25 women with cardiovascular events after pre-eclampsia

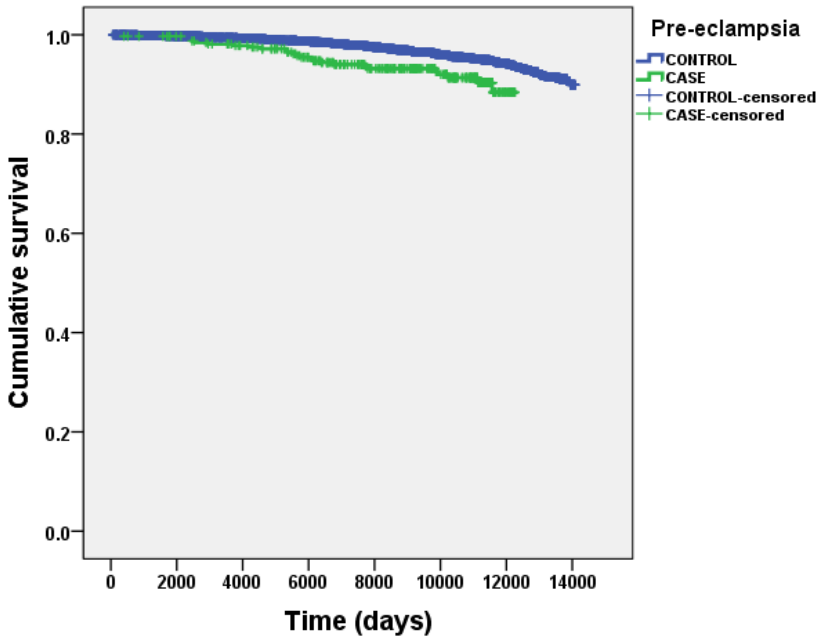


3.3.4 Survival analysis

For survival analysis, as before, index pregnancy was defined as either first pregnancy in normotensive controls, and first pre-eclamptic pregnancy (by ICD-9 or ICD-10 definition) in cases. Admission to hospital with first cardiovascular event (as defined by ICD-9 and ICD-10 coding in Table 3.9) was the endpoint of interest. In subjects who did not experience the endpoint of interest, censoring occurred either when the end of the study was reached (1st July 2013) or if they experienced death from a non-cardiovascular admission diagnosis. There were two deaths from cancer during the follow-up period, both in the normotensive group, and they were censored accordingly.

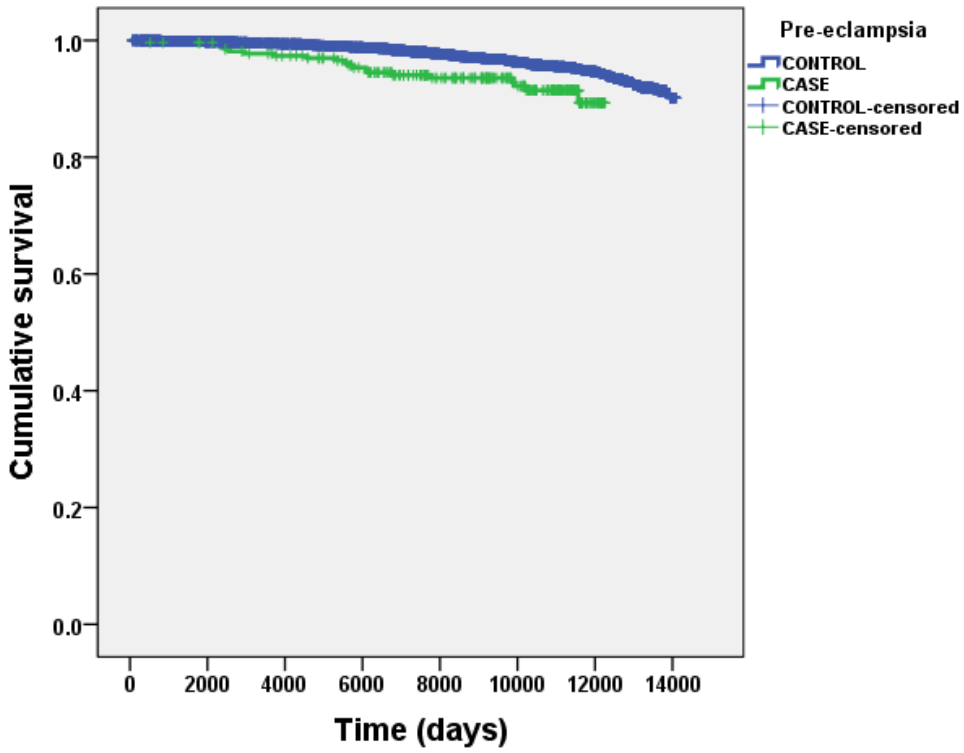
Out of a total of 5,253 women, there were 25 cardiovascular events in 331 women with pre-eclampsia (306 women were censored). There were 193 cardiovascular events in 4,922 women with normotensive pregnancies (4,729 were censored). There was a statistically significant difference between women with a history of pre-eclampsia and those with normotensive pregnancy on Kaplan-Meier survival analysis, with log rank Mantel-Cox p-value <0.001. (Figure 3.7).

Figure 3.7 Kaplan-Meier curve for time to cardiovascular event in pre-eclampsia vs normotensive pregnancies



Further survival analysis was performed after correction for possible confounders: multiple pregnancies, low birth weight (<2,500g), birth at gestation <37weeks. Data were prepared by removal of the following numbers of subjects; 277 with low birth weight (<2500g), then 107 with pre-term delivery (<37weeks gestation) then 35 with multiple gestation pregnancies. This left a total of 4,834 women (270 with pre-eclampsia and 4,564 with normotensive pregnancies). A total of 169 cardiovascular events occurred in the normotensive group, and 4,395 subjects were censored. In the pre-eclampsia group there were 20 cardiovascular events and 250 subjects were censored. Repeat survival analysis revealed the same significant results (p=0.001). Kaplan-Meier curve is seen in figure 3.8.

Figure 3.8 Kaplan-Meier curve for time to cardiovascular event in pre-eclampsia cases and normotensive controls in women following exclusion of possible confounders



3.3.5 **Cox Proportional Hazards**

In order to further investigate the effects of traditional cardiovascular risk factors on cardiovascular events, it was possible to incorporate such data, already linked through Generation Scotland. Using a Cox Proportional Hazards model, smoking, diabetes and BMI at time of recruitment to the Generation Scotland study were all found to be significantly associated with an increased risk of cardiovascular event, as was pre-eclampsia itself (see Table 3.11 for individual hazard ratios and 95% confidence intervals).

Table 3.11 Cox proportional hazards model for cardiovascular risk factors

	Hazard ratio (95% CI)	P-value
Pre-eclampsia	2.051 (1.335, 3.152)	0.001
Age (yrs)	1.004 (0.983, 1.025)	0.707
BMI (kg/m^2)	1.034 (1.011, 1.058)	0.003
SBP (mmHg)	1.004 (0.995, 1.012)	0.378
Diabetes	3.078 (1.749, 5.416)	<0.001
Current smoking	1.683 (1.220, 2.320)	0.001

On comparison of birth characteristics pre-eclampsia was found to have a two-fold increased risk of cardiovascular event (p=0.001). (Table 3.12).

Table 3.12 Cox proportional hazards model for birth characteristics

	Hazard ratio (95% CI)	P-value
Pre-eclampsia	2.001 (1.305, 3.069)	0.001
Age at index pregnancy (yrs)	1.018 (0.989, 1.048)	0.223
Birth weight at index pregnancy	1.000 (0.999, 1.000)	0.044
Gestation at delivery index pregnancy	1.004 (0.926, 1.089)	0.925
C-section	1.383 (0.967, 1.977)	0.076

3.4 Discussion

3.4.1 Findings

The main findings of this study were: 1) nulliparous women had a higher blood pressure and greater risk of cardiovascular event than parous women, 2) women with a history of pre-eclampsia had a higher blood pressure and greater risk of cardiovascular events later in life than women with normotensive pregnancies, 3) women with a history of pre-eclampsia experienced earlier gestational age at delivery and were more likely to have a caesarean section birth, and 4) on survival analysis, women with a history of pre-eclampsia were more likely to have a cardiovascular event later in life than women with normotensive deliveries. Women with a history of pre-eclampsia were found to have a lower birth weight than those with a normotensive pregnancy, however, on adjusting birth weight for gestational age the difference in birth weight was no longer significant.

3.4.2 Strengths

An important strength of this study is the use of ICD classification (which is standardised and used by most other studies in this field) and SMR01 and SMR02 data as opposed to questionnaires for information regarding cardiovascular and maternity diagnoses. This study does not depend on recall of participants many years after the event and is therefore not subject to this form of bias. Furthermore, the Scottish Information Services Division data undergo quality assessment and are therefore more likely to be reliable for retrospective studies than individual maternity record check. In addition, depending on which health board the maternity data relates to, searching through case notes of pregnancies >25 years ago can prove problematic as some records may have been destroyed.

For the purposes of this study only pre-eclamptic pregnancies were included as cases, not general hypertensive disorders of pregnancy, because they potentially differ in precise pathophysiology. Thus, rigorous exclusion of any pregnancy which was not deemed to qualify in ICD classification as pre-eclampsia was pursued. Rigorous exclusion of any "non-normotensive" pregnancy was also applied to the control group to make sure it did not incorporate any pregnancy which had any element of hypertensive disorder, mild, transient or otherwise. Other studies in this area have not consistently gone to such lengths

to exclude these milder conditions, whilst others have combined pre-eclampsia/eclampsia with other hypertensive disorders of gestation.

Another strength of this study was the ability to evaluate cardiovascular events in nulliparous women in addition to those with pre-eclampsia and normotensive pregnancies. Previous studies investigating parity and risk of cardiovascular disease and mortality later in life have provided inconsistent results (99;365;368;370-372;378-381). In the COPS record-linkage study nulliparous women had a higher risk of cardiovascular event than parous women. A recent meta-analysis of cohort studies (372) investigated parity and cardiovascular disease mortality included ten prospective studies with a total of 994,810 participants and 16,601 cardiovascular events. This study found a borderline significant reduction in risk of cardiovascular disease mortality in ever parity vs nulliparous women (RR=0.79, 95% CI: 0.60-1.06). While higher fertility may be representative of generally healthier women, and other lifestyle factors must also be taken into consideration when comparing nulliparous women with parous such as anxiety or fear of having children which might also contribute to the increased cardiovascular mortality risk in the nulliparous group (372).

Other important considerations at the outset of this work were whether to investigate data on mortality or cardiovascular events themselves. The decision to use cardiovascular events as the primary endpoint of interest extends current knowledge as to the different types of cardiovascular disease women with a history of pre-eclampsia are more likely to experience. In order to best direct future research and management strategies for cardiovascular disease prevention in women with a history of pre-eclampsia, and to be more vigilant as to what type of cardiovascular disease these women will be at greater risk of, it is important to appreciate which cardiovascular diseases these women are more likely to experience. Blood pressure was found to be higher later in life in women with a history of pre-eclampsia in comparison with normotensive women, however this information alone is not as advantageous as the additional information of which diseases of the cardiovascular system they are more likely to develop.

3.4.3 **Limitations**

This study only included women who had lived long enough to enrol in the Generation Scotland study, while women who exhibited cardiovascular events earlier in their lives and suffered a cardiovascular-related mortality are now dead. This is a form of bias. Also, these

women were participants in the Generation Scotland study. They may naturally have been healthier women or be more interested than average in their own health and general fitness and wellbeing since they actively sought to enrol in GS:SFHS.

Due to size of study it was not possible to assess the effects of recurrent pre-eclampsia, nor was it possible to separate women with only one pregnancy from women with multiple pregnancies. A recent study (382) assessed recurrent pre-eclampsia in one of the largest cohorts studied to date, with 606,820 women with two or more deliveries and 501,761 women with one delivery. Similar to the study described in this chapter, their study included both liveborn and stillborn deliveries, and only included pregnancies extending past 20 weeks (as pre-eclampsia can only be diagnosed after this time). Recurrent pre-eclampsia was associated with a higher risk of future hospitalisation with cardiovascular event and also with significantly shorter time to first event. It also found that pre-eclamptic women with only one delivery were at almost the same risk of cardiovascular hospital admission as those with recurrent pre-eclampsia.

Miscarriage has been found in the literature to itself carry a risk of cardiovascular events later in life (383). Similarly stillbirth, which has been counted as first delivery in other large studies (83) may also carry additional risk of cardiovascular events and could thus potentially bias results (383). In this population-based prospective (EPIC) cohort study of 11,518 women (in Heidelberg, Germany), mean follow-up was 10.8 years. With each miscarriage, the risk of myocardial infarction (MI) increased more than 40%. There was also a >3.5-fold increased risk of MI with a history of stillbirth.

The COPS linkage study would have been underpowered to examine the effects of different types of pre-eclampsia e.g. classification by gestational age (early onset vs late onset). However, it is important to consider how to combine studies (which have this information available) in the future, to add more power to studies investigating the possibility of different pathophysiological mechanisms of cardiovascular risk for the different classifications of pre-eclampsia which are emerging.

3.4.4 **Conclusions**

The findings of this study support an association between pre-eclampsia and later cardiovascular disease. Pre-eclampsia, along with other traditional cardiovascular risk factors such as smoking and diabetes, was found to increase the risk of future

cardiovascular disease significantly. Even in the absence of other cardiovascular risk factors, if a woman has a personal history of pre-eclampsia, she may benefit from early cardiovascular disease screening.

There is little guidance on when screening for cardiovascular disease should begin in women with a history of pre-eclampsia, or the nature of what this screening should be. Future longitudinal studies monitoring blood pressure and other non-invasive cardiovascular assessment methods may inform the likeliest age of onset of early cardiovascular disease in these women. Otherwise, it is difficult to confirm the exact length of time after pre-eclamptic pregnancy that women start to exhibit features of their increased cardiovascular risk. Obviously, having identified that these women are at increased cardiovascular risk, prevention of cardiovascular endpoints is of the utmost importance. Monitoring of the intermediate cardiovascular phenotype will be important, in order to facilitate timely intervention and prevent serious disease.

It remains clear that there is lack of consistency between epidemiological studies and their methods, therefore improved standardisation of approach would be better for future large studies and the possible integration of existing datasets.

4 The ECG in women with a history of pre-eclampsia

4.1 Introduction

The increased risk of cardiovascular disease later in life in women with a history of pre-eclampsia has several proposed mechanisms. One important finding, confirmed in the studies presented in this thesis, is that women with a history of pre-eclampsia are more likely to have a higher blood pressure later in life. In a recent study by Behrens et al (86), the risk of hypertension following hypertensive pregnancy disorders was found to be higher than in women with normotensive pregnancies in the immediate post-partum period and this difference remained and was still present at 20 years post-partum. Hypertension in pregnancy is a risk factor for adverse cardiac remodelling and in a study of women later in life (mean age 56 years), 427 women with a history of hypertensive pregnancy were compared with 2210 women with normotensive pregnancy. Those with a history of hypertensive pregnancy were found to have evidence of left ventricular hypertrophy after adjusting for age, ethnicity, BMI, hypertension, diabetes, parity and education (205). However, after the length of time of hypertension was considered, the relationship was no longer significant.

Previously held beliefs that the immediate effects of hypertensive pregnancy on the heart regressed postpartum have been challenged (202;205). In addition a study by Ray et al found evidence that at 8 years post-partum, women with a history of maternal placental syndromes (including pre-eclampsia) at index pregnancy were at an increased risk of developing heart failure and dysrhythmias than women without maternal placental syndromes (217).

Taking into consideration the above findings, possible mechanisms for left ventricular hypertrophy (LVH) following hypertensive pregnancy include longer exposure to hypertension in general in these women, or possibly the persistence of cardiac changes themselves following hypertensive pregnancy, which go unnoticed due to blood pressure settling in the immediate post-partum period.

Hypertension after pre-eclampsia has been found to be preceded by changes in cardiac structure and function in a study by Ghossein-Doha et al (201). Formerly pre-eclamptic

women who were initially normotensive but developed hypertension within the 12 years following the pregnancy were found to have had an increased left ventricular mass index and decreased diastolic dysfunction when they were screened during the post-partum screening period in comparison with normotensive formerly pre-eclamptic women.

The electrocardiogram (ECG) is a routinely used tool which can be used to detect evidence of cardiovascular risk. It has previously been used in the risk prediction of hypertensive patients (215;216;384). A study by Raffaelli et al (213) revealed that pregnant women with pre-eclampsia had a lower heart rate and significantly longer P wave duration, QT interval and QTc interval in comparison with a control group of women with normotensive pregnancies. They also had a higher QT dispersion (QTd). On further subgroup analysis, dividing pre-eclampsia into early-onset vs late-onset, there were no significant differences in any of the ECG parameters. In an earlier study by Hoogsteder et al (385), firstly, 658 women with a recent history of pre-eclampsia were screened with an ECG for any abnormalities and 23 were found to have clinically relevant findings, with 13 having evidence of ischaemic heart disease. The group then went on to evaluate whether primiparous women with early-onset pre-eclampsia, who went on to develop recurrent pre-eclampsia in a subsequent pregnancy, were different from women who had a normal second pregnancy. These women were found to have statistically significantly more leftward deviation of P-axis and R-axis and a longer QTc on ECG in comparison with women who had a normal subsequent pregnancy.

A prolonged QTc interval (generally considered to be >450ms in women) is of significance due to associated risks of arrhythmia and sudden cardiac death (386). Women, especially, are at an increased risk of life-threatening arrhythmias such as Torsades de Pointes when on medications which might prolong the QT interval (387). There are many medications which can prolong the QT interval such as antibiotics (e.g. moxifloxacin, erythromycin), antihistamines (e.g. terfenadine), antidepressants (e.g. amitriptyline) and antipsychotics (e.g. citalopram). Comprehensive lists of medications which can prolong the QT interval are available online on such websites as http://sads.org.uk/drugs-to-avoid/. Some of these websites are aimed at people with conditions such as congenital long QT syndrome, for whom the risks of adverse events are greater and who should check, before starting any medication, whether it has any effect on the QT interval. In the context of pre-eclampsia, one important medication which may prolong the QT interval is magnesium sulphate (211). This further complicates interpretation of QT interval findings in studies of QT

interval at time of delivery in women with pre-eclampsia and is mentioned as a limitation in the interpretation of data in the study by Raffaelli et al (213).

LVH itself is considered to be evidence of target organ damage in arterial hypertension and has been found to alter conduction and repolarization of the ventricle (388). In the "Losartan Intervention for Endpoint Reduction in Hypertension (LIFE)" study, in a hypertensive risk population with left ventricular hypertrophy, mortality risk was further stratified by increased QRS duration and maximum QT_{apex} interval (388). In considering determinants of QRS axis, there is evidence that as BMI increases, QRS axis shifts from rightward to leftward, and BMI should be noted when performing assessment for left ventricular hypertrophy (389).

A relatively small study by Murphy et al (390) sought to determine the effect of normal pregnancy on late trimester and postpartum ECG parameters and effects of pre-eclampsia on postpartum ECG parameters. P-wave and QRS duration were not affected by uncomplicated pregnancy, however, 20 women with a history of pre-eclampsia exhibited significantly longer P-wave and QRS duration in comparison with 15 never pregnant and 20 normotensive pregnant controls in the postpartum period (390). A persistently increased blood pressure, prolonged P-wave and QRS duration were noted at 5-10 weeks and 6-8 months after pre-eclampsia, however, after adjusting for blood pressure the differences in P-wave duration resolved. The significant QRS conduction delay at 6-8 months postpartum in women with a history of pre-eclampsia predisposes to an increased risk of cardiac arrhythmia in these women.

To date, on review of the literature by the author, no studies appear to have investigated ECG parameters in nulliparous vs parous women later in life. In Chapter 3, nulliparous women were found to be at greater risk of cardiovascular events later in life than parous women in the Generation Scotland cohort. The literature in general yields varying results regarding parity and risk of cardiovascular disease later in life. This area warrants further attention. Reasons for nulliparity vary considerably, and it is possible that different cohorts of nulliparous women in different studies are exposed to different confounders overall which are influencing the results and influencing their cardiovascular risk. It is not always possible to correct for all confounders on statistical analysis. Clinical and ECG parameters may serve to identify possible mechanisms for the increased risk identified in these women.

The aims of this study were to determine:

1) whether there was any evidence on ECG for the increased risk of cardiovascular events which was identified in nulliparous vs parous women in the Generation Scotland cohort in chapter 3.

2) whether women with a remote history of pre-eclamptic pregnancy had any evidence of LVH, atrial abnormality or increased risk of arrhythmia based on ECG parameters in comparison with women with a history of normotensive pregnancy.

4.2 **Methods**

Clinical information, maternity information and ECGs were available from Generation Scotland as part of the record linkage study described in chapter 3, with ethical approval as described in chapter 2. Here in chapter 4, the available data from the ECGs was explored further.

During the Generation Scotland study visit a 12-lead resting ECG was recorded by the Atria 6100 system (Burdick, Cardiac Science Corporation, Bothell, WA, USA). Any serious abnormalities of the ECG were followed up as necessary according to Generation Scotland study protocols. Information from the Atria 6100 was analysed centrally at the ECG core lab, Glasgow Royal Infirmary using the "Glasgow ECG analysis program". ECGs were confirmed and paper copies were sent back to the Generation Scotland co-ordinating centre. Digital results data was sent in plain text format to the Robertson Centre for Biostatistics, University of Glasgow. Information on ECG parameters, Minnesota code classifications (210), left ventricular mass index (LVMI) and measures of LVH were reported.

For the purposes of this study, heart rate, P-axis, QRS-axis, T-axis, P wave duration, QRS duration, PR interval, QT interval and QTc interval were analysed. Measures for left ventricular hypertrophy included LVMI Rautaharju (391), Cornell voltage (392), Cornell product (393) and Sokolow-Lyon criteria (394). These had already been calculated on the ECGs at the ECG core lab according to the following formulae:

LVMI Rautaharju (white women): 88.5 + 0.018(RV5) + 0.053(SV5) – 0.112(SI) + 0.108(TposV1) + 1.70(TnegaVF) – 0.094(TposV6)

Cornell voltage (female): RaVL + SV3 $\geq$ 2000 μV

Cornell product: (RaVL + SV3) x QRS duration >244 mV.ms

Sokolow-Lyon criteria: SV1 + RV5 or V6 $\geq$ 3500 μV

RV5 = amplitude of R wave in lead V5, SV5 = amplitude of S wave in lead V5, TV1 = amplitude T wave in lead V1, TaVF = amplitude of T wave in lead aVF, TV6 = amplitude of T wave in lead V6, RaVL = amplitude of R wave in lead aVL, SV3 = amplitude of S wave in V3, SV1 = amplitude of S wave in V1

The QTc intervals available from the Generation Scotland data used Hodges correction method. Hodges = QT interval +1.75 (heart rate-60).

4.2.1 Statistical analysis

Statistical analyses were performed using Minitab v17 (Minitab Inc, State College, PA, USA) and SPSS v22 (IBM Corp, Armonk, New York, USA). Normality of distribution was assessed using the Kolmogorov-Smirnov test and visual inspection of histograms and plots. Data are expressed as mean ± standard deviation unless otherwise stated. Data which were not normally distributed were transformed and non-parametric tests were used if data did not normalise on transformation. Independent samples t-test was carried out on normally distributed data and the Mann-Whitney U test was used for non-parametric data. Comparison of categorical data was performed using Chi-squared test, or Fisher's exact test as appropriate. Multiple linear regression was used to further investigate ECG parameters. A p-value of <0.05 was considered significant.

4.3 **Results**

4.3.1 **Comparison of ECGs in all GS:SFHS women: nulliparous vs parous**

There were 8820 women with ECGs available in Generation Scotland. Of them 3641 were nulliparous and 5179 had one or more children. Women who did not have children had a statistically significantly higher BMI, blood pressure and heart rate, however, numerically these differences were small (Table 4.1). They had a more leftward P-axis, QRS-axis and T-axis in comparison with parous women. P wave duration was longer and PR interval and corrected QT interval (Hodges) were also significantly longer (Table 4.2).

Table 4.1 Comparison of clinical parameters in nulliparous vs parous women in Generation Scotland

	Nulliparous (n=3641)	**Parous (n=5179)**	**P-value**
Age (yrs)	49±18	47±11	**<0.001**
BMI (kg/m^2)	26.8±5.8	26.3±5.4	**0.001**
SBP mmHg	131±19	126±17	**<0.001**
DBP mmHg	78±10	78±10	**<0.001**
HR bpm	65±10	64±10	**0.001**

All measurements are given as mean ±SD. Comparison is by independent t-test for normally distributed data and Mann-Whitney U test for non-parametric data. BMI = body mass index, SBP= systolic blood pressure, DBP= diastolic blood pressure, BW= birth weight, HR= heart rate, LVMI= left ventricular mass index.

Table 4.2 Comparison of ECG parameters in nulliparous vs parous women in Generation Scotland

	Nulliparous (n=3641)	**Parous (n=5179)**	**P-value**
P-axis	39±28	41±26	**<0.001**
QRS-axis	33±32	37±30	**<0.001**
T-axis	36±23	36±20	**0.007**
P duration (ms)	105±14	105±13	**0.019**
QRS duration (ms)	87±11	87±10	0.974
PR interval (ms)	160±25	159±23	**0.046**
QT interval (ms)	408±32	408±30	0.908
QTc Hodges (ms)	417±23	415±21	**0.007**
	(n=1826)	**(n=2806)**	
LVMI Rautaharju (g/m^2)	89.4±30.2	89.5±28.9	0.248
Cornell voltage (μV)	1550±479	1538±440	0.955
Cornell product (mV.ms)	134.6±49.7	133.9±45.3	0.745
Sokolow-Lyon (μV)	1989±589	1989±571	0.945

All measurements are given as mean ±SD. Comparison is by independent t-test for normally distributed data and Mann-Whitney U test for non-parametric data. LVMI= left ventricular mass index.

Table 4.3 Comparison of subjects meeting criteria for LVH and prolonged QTc in nulliparous vs parous women in Generation Scotland

	Nulliparous	**Parous**	**P-value**
Number fulfilling Cornell voltage criteria for LVH (>2000 μV)	272/1826 (14.9%)	372/2806 (13.3%)	0.115
Number fulfilling Cornell product criteria for LVH (>244mV.ms)	50/1825 (2.7%)	53/2803 (1.9%)	0.056
Number fulfilling Sokolow-Lyon criteria for LVH (>3500 μV)	27/1822 (1.5%)	35/2800 (1.3%)	0.503
Number with QTc >450ms	309/3638 (8.5%)	341/5172 (6.6%)	**0.001**

The number of women who fulfilled Cornell and Sokolow-Lyon criteria for left ventricular hypertrophy was greater in the nulliparous group but this was not statistically significantly different (Table 4.3). The number of women fulfilling criteria for longer QTc interval was significantly higher in the nulliparous group.

4.3.1.1 *Minnesota classifications*

There were significant differences between groups in Minnesota code classification for left bundle branch block (nulliparous 17/3439 (0.5%) vs parous 8/4893 (0.2%), p=0.007), right bundle branch block (nulliparous 20/3439 (0.6%) vs parous 9/4893 (0.2%), p=0.002) and atrial fibrillation (nulliparous 30/3439 (0.9%) vs parous 6/4893 (0.1%), p=<0.001). Other differences were not significant (Table 4.4).

Table 4.4 Minnesota code classifications in nulliparous vs parous women

Minnesota code classification	Nulliparous	Parous	P-value
IHD	272/3439 (7.9%)	381/4893 (7.8%)	0.838
LVH	28/3439 (0.8%)	24/4893 (0.5%)	0.065
Complete HB	1/3439 (0.03%)	0/4893 (0%)	0.413
Type II HB	41/3439 (1.2%)	42/4893 (0.9%)	0.131
WPW	2/3439 (0.06%)	4/4893 (0.08%)	1.000
Short PR interval	2/3439 (0.06%)	4/4893 (0.08%)	1.000
PPM	3/3439 (0.09%)	0/4893 (0%)	0.070
LBBB	17/3439 (0.49%)	8/4893 (0.16%)	**0.007**
RBBB	20/3439 (0.58%)	9/4893 (0.18%)	**0.002**
LAHB	7/3439 (0.20%)	3/4893 (0.06%)	0.104
IVCB	12/3439 (0.35%)	12/4893 (0.25%)	0.385
APC	35/3439 (1.02%)	37/4893 (0.76%)	0.204
VPC	8/3439 (0.23%)	5/4893 (0.10%)	0.137
AFIB	30/3439 (0.87%)	6/4893 (0.12%)	**<0.001**
Atrial Flutter	0/3439 (0%)	0/4893 (0%)	N/A

IHD=ischaemic heart disease, LVH=left ventricular hypertrophy, HB=heart block, WPW=Wolff-Parkinson White syndrome, PPM=pacemaker, LBBB=left bundle branch block, RBBB= right bundle branch block, LAHB=left anterior hemi-block, IVCB=intra-ventricular conduction block, APC=atrial premature complexes, VPC=ventricular premature complexes, AFIB=atrial fibrillation. P-values from Chi-squared test or Fisher's exact test as appropriate.

4.3.1.2 *Further analysis of QTc*

In order to obtain a true perspective on whether or not the QTc interval was genuinely different between groups, as many possible influences on the QT interval (confounders) were removed as possible. Any ECGs with abnormal Minnesota codes were removed and women taking any potentially QT prolonging medications were removed from the analysis.

Of the 8820 ECGs available, 945 were removed due to abnormalities on Minnesota coding, 3424 were removed because the women were on potentially QT-prolonging medication and 1536 were removed as their medication status was unknown. This left a total of 2915 women with ECGs (1252 in the nulliparous group and 1663 in the parous group) (Table 4.5).

Systolic blood pressure and heart rate were significantly higher in nulliparous women, and P-axis was significantly more leftward but there were no other significant differences in any of the ECG parameters. However, the general trends were the same as before.

Table 4.5 Further analysis following ECG exclusions

	Nulliparous women (n=1252)	**Parous women (n=1663)**	**P-value**
Age (yrs)	47±18	47±11	0.843
BMI (kg/m^2)	26.1±5.3	26.1±5.2	0.682
SBP (mmHg)	130±19	126±17	**<0.001**
DBP (mmHg)	79±10	78±10	0.411
HR (bpm)	66±10	65±10	**0.030**
P-axis (^{o})	39±27	41±27	**0.031**
QRS-axis (^{o})	36±31	36±30	0.440
T-axis (^{o})	35±21	36±20	0.271
P wave duration (ms)	105±14	104±13	0.997
QRS duration (ms)	86±9	86±8	0.410
PR interval (ms)	158±23	158±22	0.468
QT interval (ms)	405±31	407±30	0.086
QTc Hodges (ms)	415±22	415±21	0.642

All measurements are given as mean ±SD. Comparison is by independent t-test for normally distributed data and Mann-Whitney U test for non-parametric data. BMI = body mass index, SBP= systolic blood pressure, DBP= diastolic blood pressure, HR= heart rate, QTc=corrected QT interval.

4.3.2 Comparison of ECGs in women with a history of normotensive pregnancy vs pre-eclampsia

Women with a history of pre-eclampsia had a significantly higher BMI, SBP and DBP. Birth weight at index pregnancy and gestational age at index pregnancy were significantly lower (Table 4.6). Heart rate was significantly higher and QRS-axis and T-axis were different showing a more leftward axis deviation in comparison with normotensive women. QTc interval (Hodges) approached statistical significance (p= 0.054). Multiple regression

analysis was performed with QTc Hodges as the dependent variable and pre-eclampsia status and other variables which were significantly different between groups included in the analysis (age, HR, BMI, SBP, birth weight at index pregnancy, gestation at index pregnancy, QRS-axis and T-axis). Overall the model was statistically significant p=<0.001 and F=64.420. Pre-eclampsia was of borderline significance in predicting QTc Hodges while adjusting for all other variables (p=0.052), and age, HR, SBP, QRS-axis and T-axis were all statistically significant at p=<0.001. While the trend for LVMI Rautaharju and Cornell voltage were higher, these differences were not statistically significant (Table 4.7).

Table 4.6 Comparison of clinical parameters between women with a history of pre-eclamptic pregnancy and normotensive pregnancy

	Normotensive controls (n=4853)	**Pre-eclampsia (n=326)**	**P-value**
Age (yrs)	46±11	48±9	**0.005**
BMI (kg/m^2)	26.2±5.3	27.9±6.2	**<0.001**
SBP mmHg	125±17	132±16	**<0.001**
DBP mmHg	77.5±10	83±9	**<0.001**
BW at index pregnancy (g)	3385±544	3213±703	**<0.001**
Gestation at index pregnancy (wks)	39.6±1.9	38.7±2.4	**<0.001**
Age at index pregnancy (yrs)	27±5	27±5	0.331
Years since index pregnancy (yrs)	23.5±11.3	25.1±7.4	0.664

All measurements are given as mean ±SD. Comparison is by independent t-test for normally distributed data and Mann-Whitney U test for non-parametric data. BMI = body mass index, SBP= systolic blood pressure, DBP= diastolic blood pressure, BW= birth weight.

Table 4.7 Comparison of ECG parameters between women with a history of pre-eclamptic pregnancy and normotensive pregnancy

	Normotensive controls (n=4853)	**Pre-eclampsia (n=326)**	**P-value**
HR bpm	64±10	66±10	**0.001**
P-axis (^{o})	41±27	41±24	0.260
QRS-axis (^{o})	37±30	29±31	**<0.001**
T-axis (^{o})	36±20	34±21	**0.010**
P duration (ms)	104±13	105±13	0.058
QRS duration (ms)	87±10	87±11	0.409
PR interval (ms)	159±24	160±24	0.492
QT interval (ms)	408±30	408±32	0.638
QTc Hodges (ms)	415±21	418±23	0.054
	(n= 2674)	**(n= 132)**	
LVMI Rautaharju (g/m^2)	89.4±29.2	90.6±22.6	0.304
Cornell voltage (μV)	1535±440	1598±446	0.086
Cornell product (mV.ms)	133.6±45.3	139.8±44.3	0.105
Sokolow-Lyon criteria (μV)	1991±571	1950±555	0.418

All measurements are given as mean ±SD. Comparison is by independent t-test for normally distributed data and Mann-Whitney U test for non-parametric data. HR= heart rate, LVMI= left ventricular mass index.

There were no significant differences between the number of women in each group fulfilling ECG criteria for LVH. The Cornell voltage and the Sokolow-Lyon criteria denominator numbers are different for the normotensive group because the number of ECGs with that information available was different. A significantly higher number of women with pre-eclampsia fulfilled criteria for longer QTc interval (Table 4.8).

Table 4.8 Comparison of Generation Scotland subjects meeting criteria for LVH and prolonged QTc between women with a history of normotensive pregnancy vs pre-eclampsia

	Normotensive controls	**Pre-eclampsia**	**P-value**
Number fulfilling Cornell voltage criteria for LVH (>2000 μV)	348/2674 (13%)	24/132 (18.2%)	0.087
Number fulfilling Cornell product criteria for LVH (>244mV.ms)	51/2671 (1.9%)	2/132 (1.5%)	1.000
Number fulfilling Sokolow-Lyon criteria for LVH (>3500 μV)	35/2668 (1.3%)	0/132 (0%)	0.409
Number with QTc >450ms	310/4847 (6.4%)	31/325 (9.5%)	**0.027**

4.3.2.1 *Minnesota classifications*

There were no significant differences between groups on Minnesota code classification. Table 4.9 details the precise breakdown of classifications.

Table 4.9 Minnesota code classifications between women with a history of normotensive pregnancy vs pre-eclampsia

Minnesota code classification	Normotensive	Pre-eclampsia	P-value
IHD	354/4588 (7.72%)	27/305 (8.85%)	0.473
LVH	22/4588 (0.48%)	2/305 (0.66%)	0.659
Complete HB	0/4588 (0%)	0/305 (0%)	N/A
Type II HB	39/4588 (0.85%)	3/305 (0.98%)	0.744
WPW	4/4588 (0.09%)	0/305 (0%)	1.000
Short PR interval	4/4588 (0.09%)	0/305 (0%)	1.000
PPM	0/4588 (0%)	0/305 (0%)	N/A
LBBB	8/4588 (0.17%)	0/305 (0%)	1.000
RBBB	9/4588 (0.20%)	0/305 (0%)	1.000
LAHB	3/4588 (0.07%)	0/305 (0%)	1.000
IVCB	11/4588 (0.24%)	1/305 (0.33%)	0.538
APC	34/4588 (0.74%)	3/305 (0.98%)	0.500
VPC	4/4588 (0.09%)	1/305 (0.33%)	0.275
AFIB	5/4588 (0.10%)	1/305 (0.33%)	0.320
Atrial Flutter	0/4588 (0%)	0/305 (0%)	N/A

IHD=ischaemic heart disease, LVH=left ventricular hypertrophy, HB=heart block, WPW=Wolff-Parkinson White syndrome, PPM=pacemaker, LBBB=left bundle branch block, RBBB= right bundle branch block, LAHB=left anterior hemi-block, IVCB=intra-ventricular conduction block, APC=atrial premature complexes, VPC=ventricular premature complexes, AFIB=atrial fibrillation. P-values from Chi-squared test or Fisher's exact test as appropriate.

4.3.2.2 *Further analysis of QTc*

Of the ECGs in the 5179 women who had children, 510 were removed due to abnormalities on Minnesota code classification, 2073 were then removed due to potentially QT prolonging medications and 933 were removed due to unknown medication status and could therefore potentially have been on QT prolonging medication. This left a total of 1663 women with ECGs for analysis of QTc interval (Table 4.10).

Table 4.10 ECG analysis following exclusions in women with history of normotensive pregnancy vs pre-eclampsia

	Normotensive pregnancy (n=1587)	**Pre-eclampsia (n=76)**	**P-value**
Age (yrs)	47±11	49±9	0.082
BMI (kg/m^2)	26.0±5.2	28.1±5.8	**0.001**
SBP (mmHg)	126±17	135±15	**<0.001**
DBP (mmHg)	78±10	85±9	**<0.001**
HR (bpm)	65±10	66±11	0.188
P-axis (^{o})	41±27	39±22	0.314
QRS-axis (^{o})	36±30	26±27	**0.002**
T-axis (^{o})	36±20	31±18	**0.033**
P wave duration (ms)	104±13	104±14	0.539
QRS duration (ms)	86±8	86±8	0.672
PR interval (ms)	158±22	158±23	0.720
QT interval (ms)	407±30	407±32	0.951
QTc Hodges (ms)	415±21	418±21	0.277

All measurements are given as mean ±SD. Comparison is by independent t-test for normally distributed data and Mann-Whitney U test for non-parametric data. BMI = body mass index, SBP= systolic blood pressure, DBP= diastolic blood pressure, HR= heart rate, QTc=corrected QT interval.

QTc interval was longer in the pre-eclampsia group, however this was not of statistical significance. BMI and blood pressure were significantly higher in the pre-eclampsia group and QRS-axis and T-axis were also significantly different between groups.

4.4 Discussion

4.4.1 Findings

Nulliparous women had higher BMI and blood pressure than parous women, and showed a more leftward cardiac axis on ECG. They also had a significantly longer P-wave duration, PR interval and QTc than parous women.

In women with a history of pre-eclampsia in comparison with women who had a normotensive pregnancy there was also a significantly higher BMI, SBP and DBP. Birth weight and gestational age at index pregnancy were lower, heart rate was higher, and there was leftward axis deviation. QTc interval (Hodges) approached statistical significance (p= 0.054). Some of these findings are in keeping with the previous literature (213;385).

On analysis of ECG criteria for LVH, there was no statistically significant difference in the proportion of women fulfilling criteria when comparing groups based on parity or on presence or absence of pre-eclampsia. When comparing the proportion with a longer QTc (>450ms) there were statistically significant difference in both investigations, with nulliparous having a longer QTc than parous and women with a history of pre-eclampsia having a longer QTc than women with a normotensive pregnancy.

It is not possible to know when these changes developed or if they were present before or during pregnancy. On the investigation of women who were nulliparous vs parous, it seems as though pregnancy was potentially a protective factor, although it is also possible that women with better baseline health were more likely to achieve pregnancy.

4.4.2 Strengths

There were a considerable number of ECGs available for analysis and on review of the literature this study is one of the largest ECG studies relating to hypertensive disorders of pregnancy described. This study is also analysing ECGs at a longer time-point after index pregnancy (>20years) than other similar studies in the literature and the variety of information allowed for comprehensive assessment.

To the best of the author's knowledge the pre-eclampsia cases identified truly had pre-eclampsia, and the normotensive controls were normotensive. In the preparation of the data for chapter 3 any women with ICD-8 (earlier) classification of pre-eclampsia but not ICD-9 or ICD-10 classification of the condition had been excluded because it was thought that ICD-8 classification would be less reliable and they could not be "normotensive controls" if it was possible that they had experienced pre-eclampsia.

4.4.3 **Limitations**

Due to the nature of the ECG information given, and the fact that I did not personally review all paper copies of the ECGs, it was not possible to compare some ECG parameters with some of those in the literature which have been found to be of interest in women with a history of hypertensive disorder of pregnancy e.g. QT dispersion (QTd) or P-wave dispersion (Pd) (200;215).

The total number of pregnancies was not taken into account, and this could potentially have biased the results. For example, a woman with only one lifetime pregnancy has a different cardiovascular risk to a woman with more than one lifetime pregnancy. Women who only have pre-eclamptic pregnancies have different cardiovascular risks to those with a mixture of normal pregnancies and pre-eclamptic pregnancies.

There is no data on paternity of the pregnancies, so it is not known whether or not this might have contributed to the index pregnancy status or the status of subsequent pregnancies. It is also impossible to decipher the reason for the number of pregnancies women had from notes or hospital records and these decisions are personal and may be related to life-circumstances. In some women, life-threatening complications at delivery may have instigated advice that they do not have any more children, or may even have necessitated procedures such as hysterectomy which would have made it impossible to conceive again. The exact nature of "non-recurrence" of pre-eclampsia itself may have implications on cardiovascular risk.

4.4.4 **Conclusion**

ECGs in women with a history of pre-eclampsia show subtle cardiac changes which could be associated with the increased blood pressure experienced in these women. Mean age of women in this study was late forties at the time of these assessments. Future areas of study

would be to determine at what age these changes first start to evolve. When these women are described as having higher blood pressures, they are not yet within the hypertensive range, and the majority of the ECG criteria are not within the diagnostic range for evidence of LVH or risk of arrhythmia, so they would not necessarily be picked up at screening. The utility of ECG as a screening method for cardiovascular disease in these women merits further investigation.

5 Cardiovascular Consequences of Pre-eclampsia Vascular Study

5.1 Introduction

In comparison with women who had normotensive pregnancies, women with hypertensive disorders of pregnancy have a 2-fold increased subsequent risk of cardiovascular disease (6;179). There are several features common to both pre-eclampsia and cardiovascular disease, for example endothelial dysfunction. Evidence of vascular dysfunction may persist beyond pregnancy into the post-partum period, however previous studies have been inconsistent in their findings regarding whether or not these abnormalities persist for longer periods (136;142;143;395).

A recent systematic review and meta-analysis analysed markers of vascular dysfunction after hypertensive disorders of pregnancy (179). Vascular function studies had to be performed at least 3 months postpartum for the study to be included and a total of 37 studies were included in the meta-analysis. Studies assessing endothelial function using flow-mediated dilatation (FMD) showed such great heterogeneity between studies that results could not be pooled for analysis. Overall, women with hypertensive disorders of pregnancy showed evidence of arterial stiffness in comparison with women with normotensive pregnancy when assessed by augmentation index (AIx) and carotid-femoral pulse wave velocity (cfPWV) and there was also evidence of subclinical vessel atherosclerosis with a higher carotid intima-media thickness (cIMT). None of the individual studies included in the meta-analysis assessed AIx, cfPWV, cIMT and FMD all together, in the same study at the same study visit (179).

Against this background the aims of this chapter were chiefly to investigate for any evidence of subclinical vascular damage in women with pre-eclampsia vs normotensive controls and, if there were any differences found between groups, to investigate factors which might be driving these differences.

5.2 **Methods**

5.2.1 **Participant recruitment**

Patients were recruited from the Generation Scotland Scottish Family Health Study (GS:SFHS), the previous Proteomics in Pre-eclampsia (PIP) Study and from the Glasgow Western Infirmary blood pressure clinics as described in Chapter 2.

Generation Scotland participants for this study had previously been determined during the course of the record-linkage work for Chapter 3. Pre-eclampsia cases and normotensive controls had been identified and 329 cases and 658 matched controls were established for the biomarker studies described in Chapter 6. For the purposes of the COPS vascular study it was planned for these cases and controls to be invited to participate. There were 116 cases from Glasgow, 168 from Edinburgh/Dundee and 45 from Aberdeen. Although GS:SFHS was a Scotland-wide study, initial recruitment for the COPS vascular study began with the Glasgow-based cases. GS:SFHS controls based in Glasgow were also invited to participate. The Generation Scotland team sent out 1) a COPS study invitation letter, 2) a COPS study patient information sheet and 3) a copy of the COPS study consent form to all women. Based on initial recruitment from this region, GS:SFHS participants from other parts of Scotland were also invited to attend. When women attended for a study visit, their "case" or "control" status was assessed at the time of the study questionnaire, and this status was verified with Generation Scotland and anonymity was maintained. All results were concordant, and in a subgroup of the COPS study Generation Scotland cohort, maternity records were also checked for concordance with COPS study questionnaire, as discussed further in section 5.2.2.

The PIP study had recruited pregnant women from the Queen Mother's Hospital (prior to its closure), the Princess Royal Maternity Hospital, Southern General Hospital and the Ayrshire Maternity Unit. Information for previous participants who attended the BHF GCRC for clinical studies as part of the PIP study was available on the PIP study database. Eligible women were sent a COPS study invitation letter, patient information sheet and a copy of the consent form by the research nurse. The PIP study cohort were younger than GS:SFHS participants, and their pregnancies were more recent. All maternity records had been reviewed at time of recruitment to the PIP study for "case" or "control" status to be established. Any additional pregnancies in PIP study controls, which had occurred since

the end of the PIP study, might have potentially influenced the classification of these ladies for the COPS study, therefore any subsequent pregnancies in women classified as "controls" in the PIP study were reviewed as mentioned in section 5.2.3.

Recruitment from the blood pressure clinic was by the author or the COPS study research nurse who would attend the blood pressure clinics and review the lists of patients for any women who fulfilled inclusion criteria for COPS. The study was discussed with any interested women who fulfilled the inclusion criteria and they were given a copy of the invitation letter, information sheet and consent form. It was arranged that they would contact us a few days later to confirm whether or not they wished a study appointment. Patient information leaflets were also left with the blood pressure clinic nurse who was informed about the study and would pass on the information to any interested ladies if we were not able to attend the clinic. There was also interest from friends and colleagues of participants who had found out about the study by "word of mouth" and had made contact. They were sent the study information documents and were recruited to the clinic group as this best represented their age group.

In recruiting women from these groups it was hoped that various age-ranges and lengths of time since pregnancy would be available for the study. A total of 86 cases and 80 controls were recruited as follows: 46 cases and 23 controls from the GS:SFHS study, 19 cases and 20 controls from the PIP study and 21 cases and 37 controls from the blood pressure clinic group. A copy of the patient invitation letters and patient information sheets specific to each group are found in Appendices 1-9.

5.2.2 **Maternity records**

In Scotland, maternity records have a minimum retention period of 25 years since the last birth (396). It was anticipated from the outset that it might not be possible to identify and validate information for all the participants in the vascular study.

Women with a history of pre-eclampsia and controls as identified by Generation Scotland and identified through the PIP study were considered to be reliably characterised. Previous record linkage studies using SMR coding in a similar manner have shown maternity data to be reliable (376) and it has been assessed by ISD Scotland Data Quality Assurance (375). Amongst study participants recruited from the blood pressure clinic group, we requested notes for 10 cases (47.6% of cases) and 24 controls (64.9% of controls) it was possible to

review maternity records for 7 cases (70% requested) and 15 controls (62.5% requested). Concordance with maternity record obstetric history and participant self-reported obstetric history was 100%. A small proportion from the Generation Scotland group were also checked (12 cases and 12 controls were requested, n=24 in total). Review of 6 cases (50%) and 8 controls (66.7%) was possible. Concordance between hospital maternity record and participant self-reported obstetric history was 100%. In the PIP study group, 8 women had had subsequent pregnancies since the PIP study. Five of them had been in the "case" group for the PIP study, and their index pregnancies were identifiable. For the remaining 3 women, who had been in the "control" group for PIP, they reported no symptoms or birth characteristics suggestive of pre-eclampsia and their "control" status for the COPS study was confirmed on review of their maternity records.

5.2.3 **Definition of index pregnancy**

The index pregnancy was defined in the same way as it had been for the record-linkage study in Chapter 3. Miscarriages or pregnancy losses at <20 weeks were excluded as index pregnancies, because pre-eclampsia can only be diagnosed beyond 20 weeks gestation. In the "control" group, the index pregnancy was the first pregnancy. If the first pregnancy fulfilled the above exclusion, the first pregnancy which continued past 20 weeks was used instead. In the "case" group, the first pregnancy fulfilling ISSHP guidelines for pre-eclampsia (described in Chapter 1) with "2+" protein on dipstick was used as the index pregnancy. Cases of haemolysis, elevated liver enzymes and low platelets (HELLP) and eclampsia were labelled as pre-eclampsia for the purposes of this study.

5.2.4 **The COPS Vascular Study**

Patients who fulfilled inclusion criteria for the study gave written informed consent. Study participants were invited either in the morning or the afternoon, depending on availability, and time of study visit was noted. Subjects were asked to refrain from caffeine, alcohol or smoking for 6 hours before the test and have only a light meal. The study visit is described in more detail in Chapter 2. It began with a questionnaire including obstetric history (Appendix 10) and height and weight measurements. Blood pressure measurements were taken and blood and urine samples obtained. A standard 12-lead ECG was also performed. Individual vascular studies were carried out in the following order: pulse wave analysis, pulse wave velocity, carotid ultrasound and flow-mediated dilatation (Figure 5.1). Study

visits were carried out either by myself or by research nurse Joanne Flynn who was fully trained in all aspects of the COPS study.

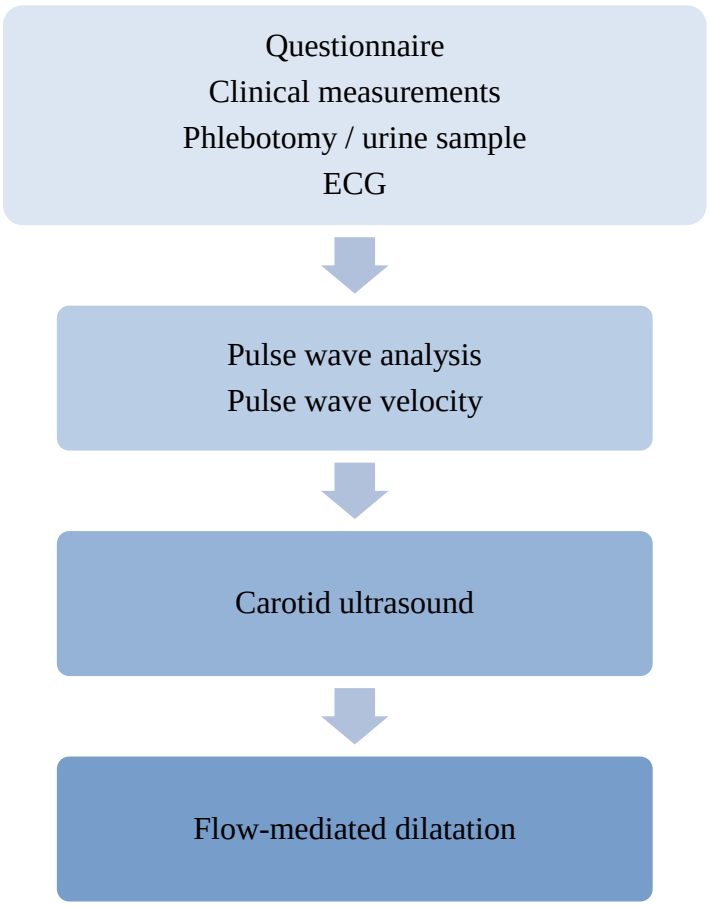


Figure 5.1 COPS Vascular study protocol detailing the order of tests.
ECG - electrocardiogram

5.2.5 Study visit

All clinical vascular studies were carried out at the British Heart Foundation Glasgow Cardiovascular Research Centre (BHF GCRC).

5.2.6 Vascular studies

5.2.6.1 *Training*

Before the study commenced both the author and research nurse (JF) had trained for over 4 months in the vascular techniques as part of a previous study "Target organ damage in predicting cardiovascular risk" which used the same standard operating procedures. More

intensive training in carotid ultrasound scanning was also provided at the Department of Vascular Medicine, Academical Medical Centre, Amsterdam with Mr Johan Gort and Dr Eric de Groot.

5.2.6.2 *COPS vascular studies*

Supine blood pressure was measured using the same calibrated oscillometric device (Omron 705-IT) in the same arm as sitting blood pressure. Participants were asked to refrain from talking during the study.

5.2.6.3 *Pulse wave analysis*

PWA was performed using applanation tonometry with the SphygmoCor® system (AtCor Medical, Sydney, Australia). The tonometer was connected to an electronics module and peripheral pulse pressure waveform at the right radial artery. Subjects were rested in a supine position and the wrist was slightly dorsiflexed. Using the tonometer (Millar Instruments, Houston, Texas, USA) gentle pressure was applied to the area of maximal pulsation of the radial artery while the wrist was supported. Tonometer position was adjusted until the best quality waveforms were seen and a 10 second recording of pulse pressure tracings was made. The SphygmoCor® system utilises software which calculates the aortic pulse waveform and aortic systolic and diastolic blood pressures using a validated transfer function. The tracings were then inspected on the SphygmoCor® software (Figure 5.2). Tracings should exhibit an initial sharp upstroke, rising to an initial peak, a second shoulder, and a notch marking the closure of the aortic valve. If these features were not present the tonometer was repositioned and further waveform recordings obtained until the relevant features were seen. Quality control indices were also checked. These consisted of pulse height variation <5%, diastolic variation <5%, shape variation <5%, average pulse height >100units. These contributed to the overall operator index which was only accepted if >80%. The augmentation index was also adjusted to a heart rate of 75 beats per minute (AIx@HR75) by the software. This was noted along with the aortic systolic and diastolic blood pressure readings. PWA readings were made in duplicate and an average of the two readings used in subsequent analyses.

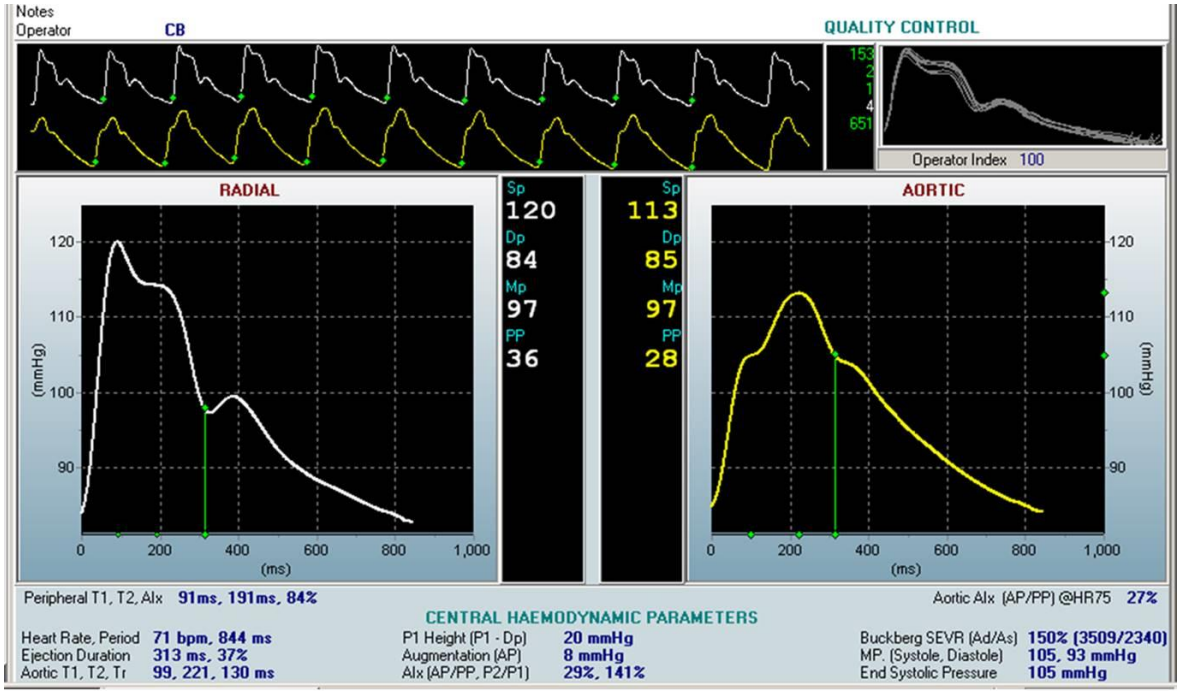


Figure 5.2 Augmentation index on pulse wave analysis

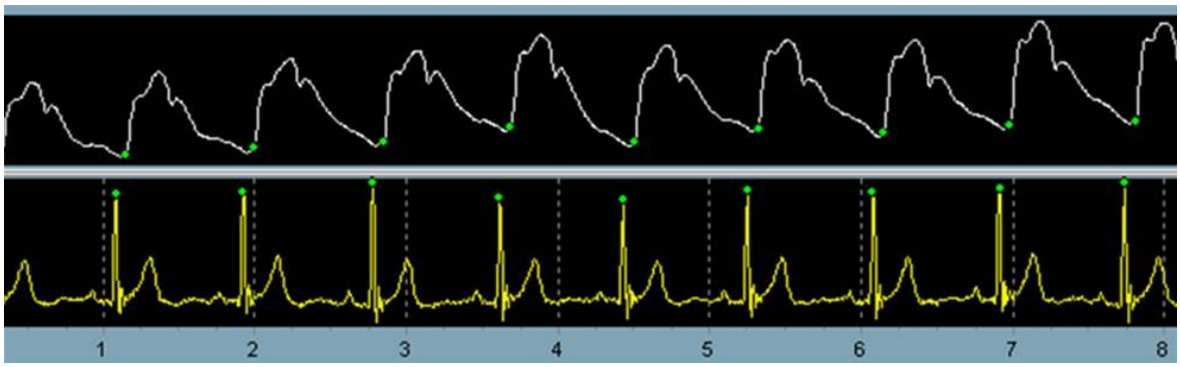
This is the augmentation index measurement for COPS study participant C-00152-C. On the left is the radial waveform in white and on the right is the central waveform. The operator index is 100%. Augmentation index at the bottom of the screen is 29%, and 27 % after adjusting for heart rate of 75bpm (see value 27% under the picture of the central waveform).

5.2.6.4 *Pulse wave velocity*

Carotid-femoral PWV (cfPWV) is considered to be the "gold standard" measurement of arterial stiffness (162). Examples of tracings in a COPS study subject are shown in Figure 5.3. Pulse wave velocity was calculated using the foot-to-foot flow wave velocity technique (162) and is the distance: transit time ratio. It is calculated using the following equation: PWV (m/s) = distance (metres)/time (seconds). Distance is "travel distance" which is the subtracted distance (sternum-carotid distance subtracted from the sternum-femoral distance).

The SphygmoCor® Vx device (AtCor Medical, Sydney, Australia) was used to measure carotid-femoral pulse wave velocity (cfPWV) non-invasively in the supine position. Three ECG leads were attached to the chest and the tracing was inspected to ensure sinus rhythm. This ECG gating on the R-wave permits the time lapse between pulse waves at the proximal and distal sites to be measured sequentially. Applanation tonometry was performed using a high-fidelity tonometer (Millar Instruments, Houston, Texas, USA). The onset of the pressure pulse was measured using the "intersecting tangent method" (397).

A Carotid



B Femoral

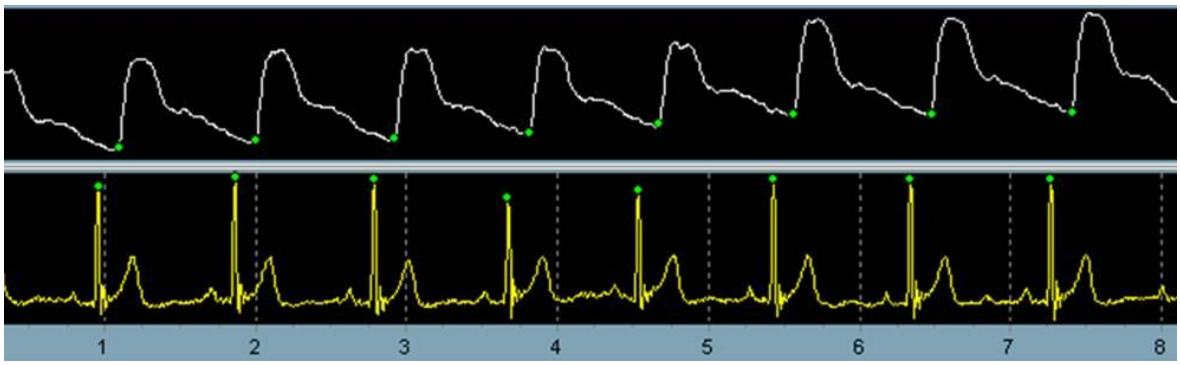


Figure 5.3 Pulse wave velocity
PWV tracings from A) the carotid and B) the femoral artery from COPS participant C-00152-C are shown.

Distal measurements were made at the site of maximal pulsation of the femoral artery and proximal measurements were made at the site of maximal pulsation of the carotid artery. The distance between right carotid pulsation and the suprasternal notch was measured in millimetres. The right femoral artery was then located at its point of maximal pulsation, and the distance from the suprasternal notch to femoral artery (via the umbilicus) was measured in millimetres.

PWV studies were recorded for up to 15 seconds at a time, and at least two readings meeting quality control criteria were recorded. Readings should have a standard deviation of <10%. An average of the two readings was used in later analysis.

5.2.6.5 *Carotid intima-media thickness measurements (cIMT)*

Carotid intima-media thickness (cIMT) was assessed using Acuson Sequoia 512 ultrasound scanner (Siemens, Erlangen, Germany). An 8L5, 5-8 MHz linear-array transducer, was

used in B-mode at a depth of 40mm and frequency 8MHz. Still images and dynamic clips were recorded at the distal common carotid artery (CCA), carotid bulb and internal carotid artery (ICA) on both the right and left sides at an ear-to-ear angle in accordance with recommendations from the Vascular Imaging laboratory at the Academic Medical Centre, Amsterdam, Netherlands (Figure 5.4). ECG was recorded simultaneously to enable images to be measured on the R wave. The mean of the right and left readings were used as the measurement for each of the three sections imaged. Internal carotid artery Doppler velocity was measured (in metres per second) to assess for significant stenosis, and readings >1.25m/s were referred for a follow-up scan and further assessment if required. In the COPS vascular study 2 subjects were referred on for further assessment.

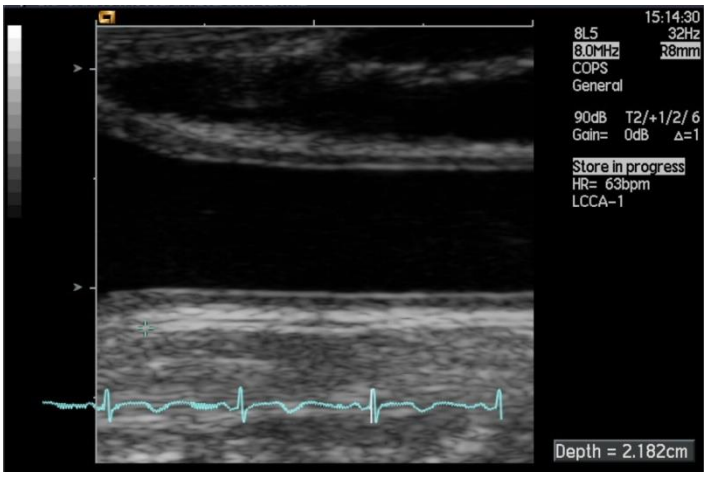
Each scan was saved as a DICOM (Digital Imaging and Communications in Medicine) file. Images were measured offline using the Syngo Workplace Siemens Arterial Health Package 3.5 (Siemens Medical Solutions USA Inc. CA). Measurements were made on a 1cm length of the far wall of the common carotid, bulb and internal carotid arteries. All scans were analysed by the author, blinded to the subject's ID code and case/control status.

The same software was also used to measure the plaque score. A plaque was defined according to the Mannheim consensus (181;182) as:

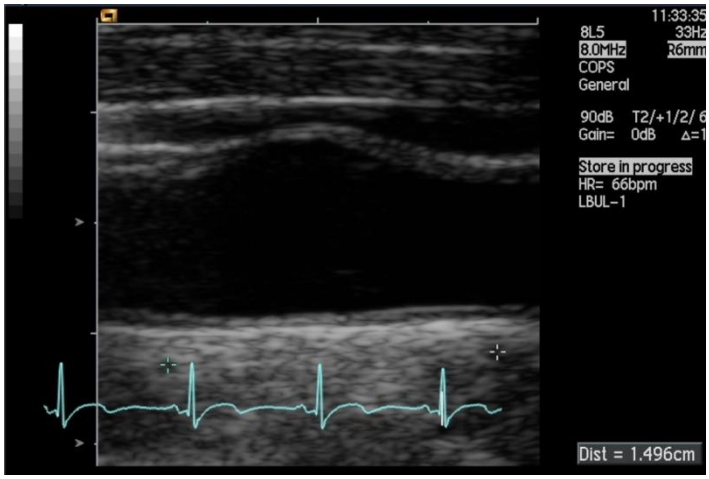
> "a focal structure encroaching into the arterial lumen of at least 0.5mm or 50% of the surrounding carotid intima-media thickness (cIMT) value, or demonstrating a thickness >1.5mm as measured from media-adventitia interface to intima-lumen interface."

The plaque score itself was calculated according to a previously defined equation (398) such that the number of sites with plaque detected (from the right and left CCA, bulb and ICA) was divided by the total number of sites with analysable images, then multiplied by six. It was not possible to assess plaque volume using the software available at time of analysis.

A



B



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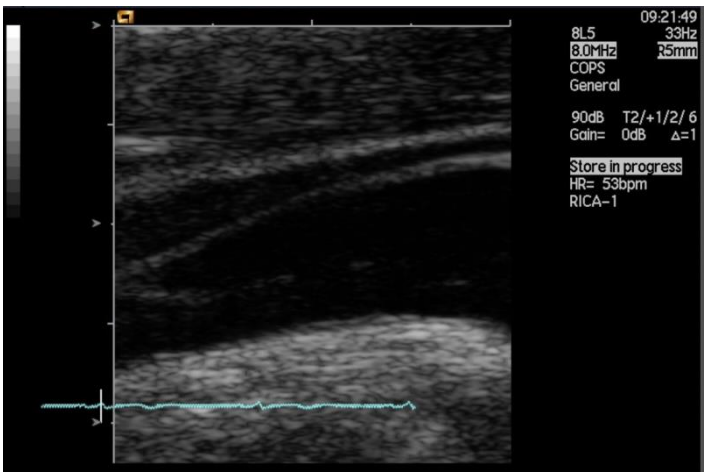

Figure 5.4 Carotid ultrasound images

A) the common carotid artery (CCA), B) the carotid bulb C) the internal carotid artery (ICA).

5.2.6.6 *Flow-mediated dilatation*

Flow-mediated dilatation (FMD) was measured with the subject supine using Doppler ultrasound at the brachial artery. A blood pressure cuff was applied to the forearm, distal to the artery. The brachial artery was identified scanning longitudinally using the Siemens Sequoia 512, 8L5 linear array transducer (the same transducer as for cIMT measurement), set at 8MHz, a depth of 30mm, and positioned at 5-10cm proximal to the antecubital fossa using a stereotactic clamp. Simultaneous electrographic recordings were made and all images were triggered on the R wave. Baseline artery diameter was measured at end-diastole.

Baseline images were recorded for 3 minutes. The forearm blood pressure cuff was then inflated to supra-systolic pressures (at least 200mmHg or 50mmHg above systolic blood pressure if this was higher) for 5 minutes. The cuff was released and recordings of the scanned artery were made for 5 more minutes. Four women did not have the test performed due to a history of Raynaud's and five women had FMD performed on the left arm due to previous breast surgery on the right side.

Images were analysed offline using software Brachial Analyzer, Medical Imaging Applications LLC, Coralville, IA, USA (Figure 5.5). All images were analysed by one reader (the author), blinded to the subject's ID case/control status. The flow-mediated dilatation is expressed as the percentage change in arterial diameter pre and post cuff occlusion relative to the baseline diameter. A "region of interest" ROI box was placed over the image at a region where near and far walls of the brachial artery were visualised. Automated software determined the vessel borders and the analysis was run. All images were reviewed and excluded if confidence interval for the measurement was less than 70%. The baseline diameter was calculated as an average of the baseline images 1 minute prior to cuff inflation. Maximum diameter (or "peak" diameter) was taken as the average of the highest reading and the two readings immediately on either side of it (three readings in total).

A) B)

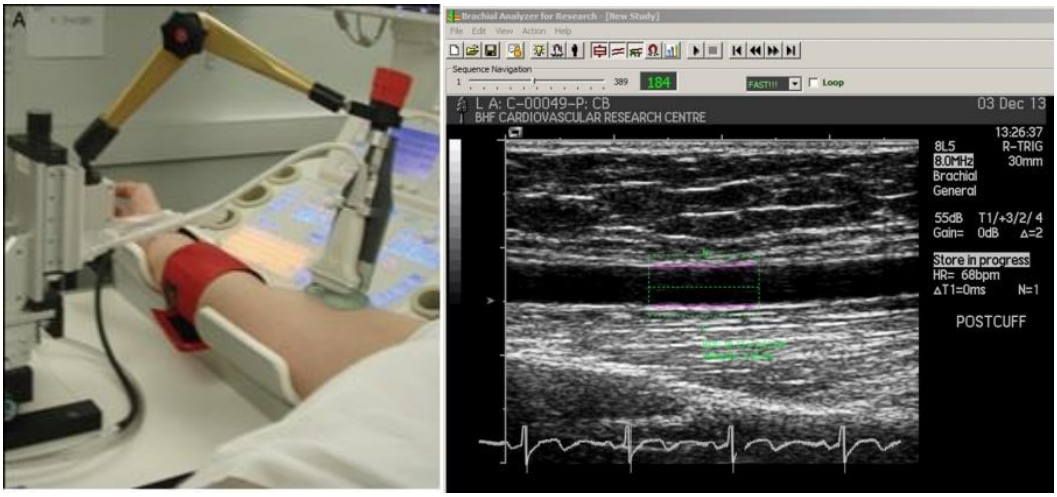

Figure 5.5 Flow-mediated dilatation images

A) Position of inflation cuff over forearm and positioning of ultrasound probe over the brachial artery (from Charakida et al (113) with permission) B) Brachial Analyzer software measuring the brachial artery diameter using a region of interest (ROI) box.

5.2.6.7 *Intra-operator and inter-operator assessment*

Because all study measurements were performed either by the author or a trained research nurse (JF), it was important to assess for intra-and inter-operator differences in the more operator-dependent studies: carotid ultrasound and flow-mediated dilatation.

For carotid ultrasound intra-observer coefficient of variation was <6% for both the author and research nurse in all 3 sections of the carotid artery imaged. Mean absolute differences were <0.055mm. These results are well within the recommendations by the American Society of Echocardiography (180). They also comply with the recommendation of mean absolute difference <0.15mm from the Department of Vascular Medicine, Academic Medical Centre, Amsterdam. Intra-class correlation coefficient between operators was 0.955 (95% CI 0.839-0.988) at the CCA, 0.984 (95% CI 0.926-0.996) at the carotid bulb and 0.944 (95% CI 0.790-0.986) at the ICA.

Twenty-five cIMT scans were picked at random for repeat measurement to assess reader reproducibility of the author. Scans were read in a blinded fashion. Results can be seen in Table 5.1.

Table 5.1 Reader reproducibility for carotid IMT measurements

COPS study ID	Mean of right and left CCA (mm)	Mean of right and left bulb (mm)	Mean of right and left ICA (mm)	Repeat mean of right and left CCA (mm)	Repeat mean of right and left bulb (mm)	Repeat mean of right and left ICA (mm)
C-00006-P	0.5500	0.5675	0.4080	0.5305	0.5445	0.4280
C-00011-P	0.6780	0.5745	0.5660	0.6610	0.5550	0.5430
C-00018-C	0.6955	0.5945	0.4245	0.6840	0.5810	0.4490
C-00035-P	0.5620	0.5125	0.3180	0.5795	0.5075	0.3070
C-00038-P	0.5790	0.5795	0.4510	0.5920	0.5695	0.4775
C-00044-P	0.5070	0.6255	0.4005	0.5160	0.6200	0.3980
C-00049-P	0.5630	0.6050	0.5760	0.5390	0.5925	0.5420
C-00060-G	0.4705	0.5495	0.4845	0.4750	0.5420	0.4655
C-00063-G	0.8080	0.7770	0.6120	0.7930	0.7700	0.5815
C-00073-C	0.6180	0.5445	0.3475	0.6335	0.5310	0.3690
C-00081-C	0.5495	0.6765	0.3845	0.5700	0.6875	0.3685
C-00085-G	0.5825	0.6325	0.5735	0.5835	0.6470	0.5690
C-00114-G	0.7085	0.7105	0.7120	0.6670	0.7025	0.6560
C-00118-G	0.5535	0.5455	0.3965	0.5460	0.5475	0.4025
C-00120-C	0.5155	0.6355	0.5350	0.5000	0.6565	0.5110
C-00126-C	0.6000	0.5900	0.5570	0.5860	0.5740	0.5325
C-00129-G	0.6480	0.5805	0.5050	0.6545	0.5950	0.4955
C-00133-G	0.7620	0.7470	0.5575	0.7500	0.7365	0.5685
C-00136-C	0.6010	0.9240	0.4600	0.6190	0.9035	0.4760
C-00137-G	0.5575	0.6395	0.6365	0.5490	0.6195	0.6455
C-00142-G	0.6050	0.7055	0.5560	0.5860	0.7135	0.5260
C-00147-C	0.6275	0.6890	0.5555	0.6375	0.6685	0.5295
C-00159-C	0.5750	0.5660	0.4100	0.5565	0.5870	0.4300
C-00162-C	0.5695	0.5125	0.5790	0.5975	0.4975	0.6045
C-00165-C	0.5535	0.5685	0.5180	0.5795	0.5720	0.4840

Intraclass correlation coefficient				
	ICC	**(95% CI)**	**F**	**P-value**
CCA	0.973	(0.940-0.988)	0.360	0.554
Bulb	0.988	(0.972-0.995)	0.383	0.063
ICA	0.967	(0.927-0.985)	1.977	0.173

CCA= common carotid artery, ICA= internal carotid artery,

For flow-mediated dilatation the intra-observer coefficients of variation were <10% for both the research nurse and the author, and intra-class correlation coefficient between operators was >0.900 for both operators.

Twenty-five FMD scans were again picked at random to be re-read (blinded) for reader reproducibility assessment as detailed in Table 5.2.

Table 5.2 Reader reproducibility for flow-mediated dilatation measurements

COPS study ID	Baseline diameter (mm)	Maximum diameter (mm)	FMD (%)	Repeat baseline diameter (mm)	Repeat maximum diameter (mm)	Repeat FMD (%)
C-00015-P	3.79	3.99	5.3	3.84	4.05	5.47
C-00017-P	2.74	2.99	9.1	2.79	2.99	7.17
C-00018-C	5.3	5.53	4.3	5.29	5.52	4.3
C-00022-P	3.06	3.24	5.9	3.09	3.25	5.2
C-00034-P	3.3	3.46	4.8	3.29	3.46	5.2
C-00047-C	3.3	3.62	9.7	3.27	3.55	8.56
C-00049-P	3.58	3.91	9.2	3.58	3.90	8.94
C-00050-G	4.74	4.83	1.9	4.73	4.81	1.7
C-00060-G	4.02	4.12	2.5	4.03	4.10	1.7
C-00068-G	3.52	3.7	5.1	3.49	3.68	5.44
C-00072-G	3.68	4.15	12.8	3.69	4.14	12.2
C-00085-G	4.11	4.34	5.6	4.12	4.38	6.3
C-00092-C	3.81	4.09	7.3	3.80	4.07	7.1
C-00101-G	3.59	3.75	4.5	3.61	3.75	3.88
C-00105-G	4.5	4.6	2.2	4.48	4.59	2.46
C-00115-G	4.76	4.83	1.5	4.77	4.85	1.68
C-00121-G	4.12	4.39	6.6	4.13	4.40	6.54
C-00128-G	3.98	4.2	5.5	3.97	4.21	6.05
C-00131-C	3.78	4.07	7.7	3.79	4.08	7.65
C-00139-G	4.01	4.13	3.0	4.00	4.13	3.25
C-00140-G	3.71	3.86	4.0	3.72	3.89	4.57
C-00146-G	4.11	4.2	2.2	4.10	4.18	1.95
C-00147-C	3.78	4.15	9.8	3.78	4.13	9.26
C-00153-C	4.21	4.42	5.0	4.24	4.42	4.25
C-00158-C	3.78	3.91	3.4	3.79	3.88	2.37

Intraclass correlation coefficient				
	ICC	**(95% CI)**	**F**	**P-value**
Baseline diameter (mm)	0.999	(0.998-1.00)	1.115	0.301
Maximum diameter (mm)	0.999	(0.997-1.00)	0.307	0.584
FMD %	0.973	(0.938-0.988)	3.362	0.079

FMD = flow-mediated dilatation

5.2.7 Statistical analysis

Coefficients of variation described in this chapter were calculated using MedCalc Statistical Software version 17.5 (MedCalc Software bvba, Ostend, Belgium). The Venn diagram (Figure 5.6) was drawn using Venny 2.1 software (399). Other statistical analysis was performed using IBM SPSS Statistics for Windows version 22 (Armonk, NY, USA: IBM Corp), Minitab version 17 (Minitab Inc, State College, PA, USA). Normality of data distribution was assessed using the Kolmogorov-Smirnov test. All data that were not normally distributed were transformed. Data are described in the tables for this chapter as mean ± standard deviation unless otherwise stated. Comparisons were made using independent t-test for normally distributed data and Mann-Whitney U test for non-parametric data. Chi-squared test or Fisher's exact test were used to compare categorical variables. Further comparisons were made using univariate and multivariate analysis. A p-value of <0.05 was considered to be significant.

5.3 Results

5.3.1 Comparisons between index pregnancies with pre-eclampsia and normotensive controls

There were 80 normotensive controls and 86 women with a history of pre-eclampsia recruited. Descriptive statistics for baseline results from clinical examination, questionnaire and lipid results are shown in Table 5.3.

Study participants were matched as closely as possible for age at time of recruitment and overall this result was not significantly different between cases and controls. There was a significant difference in weight, BMI, SBP, DBP and current diagnosis of hypertension between groups.

Table 5.3 Results for all 166 COPS study participants

	Normotensive Controls (N=80)	**Pre-eclampsia Cases (N=86)**	**P-value**
Age at study visit (yrs)	48.8±8.5	47.6±10.1	0.406
Height (cm)	162.7±6.7	161.4±6.5	0.193
Weight (kg)	70.1±11.2	76.4±15.0	**0.004**
BMI (kg/m^2)	26.6±4.5	29.4±6.1	**0.001**
Mean* SBP sitting (mmHg)	123±10	130±14	**<0.001**
Mean* DBP sitting (mmHg)	78±7	83±8	**<0.001**
Resting HR (bpm)	71±9	73±11	0.123
SBP supine (mmHg)	119±11	126±15	**<0.001**
DBP supine (mmHg)	74±8	77±9	**0.010**
Total cholesterol (mmol/L)	5.4±1.0	5.3±1.0	0.538
Triglycerides (mmol/L)	1.36±0.72	1.35±0.71	0.941
HDL (mmol/L)	1.52±0.33	1.49±0.34	0.761
Non-HDL cholesterol (mmol/L)	3.85±0.10	3.86±0.99	0.986
Current diagnosis of hypertension	7 (8.8%)	26 (30.2%)	**0.001**
Current diagnosis of diabetes	0 (0%)	5 (5.8%)	0.060
Current smoker	11(13.8%)	7 (8.1%)	0.245

Data are presented as mean ± SD for continuous data or as a proportion for categorical data. BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, HDL = high density lipoprotein. * Mean of the 2nd and 3rd blood pressure readings taken.

For those participants with a diagnosis of hypertension, the breakdown of specific antihypertensive medications taken is shown below in Table 5.4. There were 1/7 (14.3%) participants on more than two antihypertensives in the normotensive pregnancy group and 6/26 (23%) in the pre-eclamptic pregnancy group. Fisher's exact test revealed no difference (p=value 1.000). The largest number of antihypertensive medications simultaneously being taken was four.

Table 5.4 Frequencies of antihypertensive medications

Antihypertensive medication groups	**Normotensive pregnancy controls with current diagnosis of hypertension (N=7)**	**Pre-eclampsia pregnancy cases with current diagnosis of hypertension (N=26)**
ACE inhibitor	2	11
Beta-blocker	2	5
Calcium channel blocker	1	6
Diuretic	3	10
Angiotensin-II receptor antagonist	2	9
Alpha-adrenoceptor blocker	0	1
Total number of medications	10	42

ACE = Angiotensin converting enzyme

Analysis of birth characteristics as shown in Table 5.5 revealed a significantly lower gestational age at delivery and a significantly lower birth weight in women with a history of pre-eclampsia in comparison with normotensive controls. For twin pregnancies, the mean of the two birth weights was used as the overall birth weight for that pregnancy. After adjusting birth weight for gestational age using the z-score, there was no longer a statistically significant difference although the trend still reflected a lower birthweight in women with a history of pre-eclampsia.

Table 5.5 Comparison of birth characteristics of index pregnancy

	Normotensive Controls (N=80)	**Pre-eclampsia Cases (N=86)**	**P-value**
Liveborn singleton pregnancy	78 (97.5%)	81(94.2%)	0.445
Liveborn twin pregnancy	2 (2.5%)	4 (4.7%)	0.683
Stillbirth	0	1 (1.1%)	1.000
Age at index pregnancy (yrs)	30±6	29±6	0.226
Time since index pregnancy at study visit (yrs)	19.2±10.9	19.2±10.2	0.933
Gestation at delivery (wks)	39.7±2.1	37.7±3.2	**<0.001**
Birthweight (g)	3397±530	2996±716	**<0.001**
Birthweight z-score	-0.203±0.853	-0.286±0.843	0.536
C-section	19/80 (23.8%)	29/86 (33.7%)	0.157

Data are presented as mean ± SD for continuous data or as a proportion for categorical data.

In order to visualise the links between pregnancies with higher risk features (such as low birth weight, gestational age at delivery <37weeks, C-section), a Venn diagram was constructed as seen Figure 5.6. Out of all index pregnancies there were a total of 86 cases of pre-eclampsia, 23 deliveries with birthweight <2500g, 26 preterm deliveries and 48 women requiring C-section. The total number of women represented by these four outcomes combined was 108.

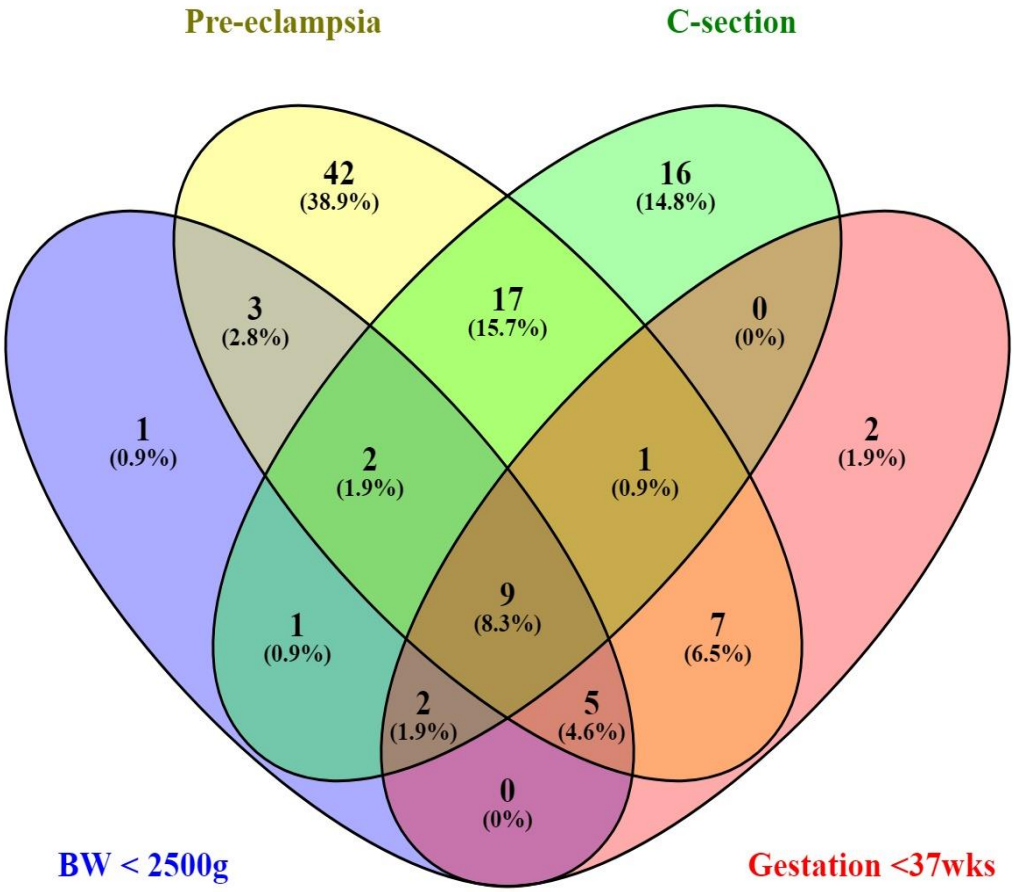

Figure 5.6 Venn diagram of outcome of index pregnancies

Data are represented as the count per section e.g. 17 women experienced pre-eclampsia and had a C-section but did not have a low birth weight baby (<2500g) or a pre-term delivery (<37 weeks gestation). Percentages shown are the proportion of women represented by the count in that particular section in comparison with the total count of women who experienced low birth weight, pre-eclampsia, C-section or pre-term delivery. No woman is counted twice.

BW = birth weight.

5.3.2 **Summary of all pregnancies**

When the information from all pregnancies in all women was assessed and combined (Table 5.6) there were a total of 217 pregnancies in the 80 women in the control group and 243 pregnancies in the 86 women in the pre-eclampsia group. Of the 243 pregnancies in the pre-eclampsia group, 105 were diagnosed with pre-eclampsia and an additional 21 were diagnosed with pregnancy-induced hypertension. Controls did not experience pregnancy induced hypertension in any pregnancy. There was a significant difference in gestation at delivery and birth weight between groups. Gestation at delivery, and birth weight were significantly lower in the pre-eclampsia group (Figure 5.7) however this difference was no longer significant on adjustment of birth weight for gestational age using z-scores. The number of C-section deliveries was not statistically different between groups with 41 (18.9%) of controls vs 50 (20.6%) cases, p=0.737.

Table 5.6 Comparison of birth characteristics for all pregnancies

	Normotensive Controls N=217 pregnancies (in 80 women)	**Pre-eclampsia Cases N=243 pregnancies (in 86 women)**
Singleton livebirth pregnancies	166 (76.5%)	184 (75.7%)
Multiple gestation livebirth pregnancies (twins)	3 (1.4%)	5 (2.0%)
Stillbirth	1(0.5%)	6 (2.5%)
Miscarriage/loss at <20 weeks	47 (21.7%)	48 (19.8%)
Breakdown by number of pregnancies per woman		
Only 1 pregnancy	7	12
Total of 2 pregnancies	40	31
Total of 3 pregnancies	18	21
Total of 4 pregnancies	6	12
Total of 5 pregnancies	5	4
Total of 6 pregnancies	3	5
Total of 7 pregnancies	0	0
Total of 8 pregnancies	0	1
Total of 9 pregnancies	1	0

Data shown in the top half of the table are as proportions of all pregnancies in that group, e.g. there were 166 livebirths out of 217 pregnancies. The lower half of the table describes the count of women who had the number of pregnancies detailed in each group.

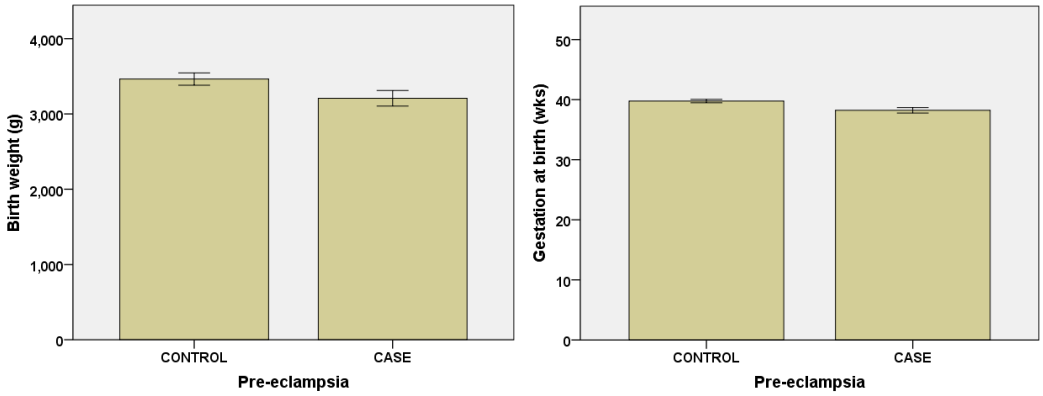

Figure 5.7 Bar charts of mean birth weight and mean gestational age at delivery for all pregnancies. Error bars for 95% confidence interval are shown.

The significantly lower birth weight and gestational age at delivery in women with pre-eclampsia was present not only for the index pregnancy, but for all pregnancies. In order to determine whether there was some pre-disposition for subclinical problems in other pregnancies which were non-hypertensive, but occurred in the pre-eclampsia cases group, the data were further explored as detailed in Table 5.7. First, the original data for all pregnancies were compared again with twin pregnancies removed, as these are more likely to have lower birth weights and earlier gestation at delivery and could potentially have biased the results (there were 3 twin pregnancies in the control group and 5 in the pre-eclampsia group). Next, the original dataset was re-examined with all index pre-eclamptic pregnancies in the “cases” group and all other pre-eclamptic or hypertensive pregnancies outwith the index pregnancy also removed. This analysis was also repeated with twin pregnancies removed in case of bias (there were 3 twin pregnancies in the control group but no twin pregnancies were left in the cases group).

Table 5.7 Further analysis of birth weight and gestational age in livebirths

	Normotensive controls	**Pre-eclampsia cases**	**P-value**
All livebirth pregnancies	**n=169**	**n=189**	
Gestation at delivery (wks)	39.76 ± 1.85	38.45 ± 2.79	**<0.001**
Birth weight (g)	3488.4 ± 500.3	3226.0 ± 707.3	**<0.001**
Birth weight z-score	-0.076 ± 0.818	-0.086 ± 0.893	0.914
All livebirth pregnancies above excluding twin pregnancies	**n=166**	**n=184**	
Gestation at delivery (wks)	39.85 ± 1.70	38.53 ± 2.75	**<0.001**
Birth weight (g)	3513.8 ± 461.5	3252.6 ± 692.5	**<0.001**
Birth weight z-score	-0.060 ± 0.818	-0.0645 ± 0.8911	0.965
All livebirth pregnancies excluding all pre-eclampsia and pregnancy-induced hypertension	**n=169**	**n=63**	
Gestation at delivery (wks)	39.76 ± 1.85	39.88 ± 1.81	0.884
Birth weight (g)	3488.4 ± 500.3	3576.1 ± 606.3	0.360
Birth weight z-score	-0.076 ± 0.818	0.1035 ± 0.999	0.207
All livebirth pregnancies above excluding twin pregnancies	**n=166**	**n=63**	
Gestation at delivery (wks)	39.85 ± 1.70	39.88 ± 1.81	0.729
Birth weight (g)	3513.8 ± 461.5	3576.1 ± 606.3	0.271
Birth weight z-score	-0.060 ± 0.818	0.1035 ± 0.999	0.167

Data are presented as mean ± SD.

The significant difference in gestation at delivery and birth weight remained between groups even with the removal of twin pregnancies. However, after removing all cases of pre-eclampsia (and pregnancy-induced hypertension) in any pregnancy from the "cases" group, there was no significant difference in the comparison of birth weight or gestation at delivery for normotensive pregnancies in cases vs control pregnancies. There was no difference in birth weight z-score in any of the comparisons.

5.3.3 Results of vascular studies

Women with a history of pre-eclampsia had a significantly higher heart-rate adjusted augmentation index (AIx@HR75), mean arterial pressure (MAP), and central SBP and DBP compared with controls (Table 5.8). PWV was also greater in cases vs controls. FMD was lower in cases than controls. On further analysis of FMD itself, baseline brachial artery diameter and post-inflation diameter were similar. There was no difference in cIMT

however there was a statistically significant difference in plaque score between groups. Figures 5.8 and 5.9 show graphical presentations of these findings. In the control group 31.6% women had at least one carotid plaque and 51.2% of women with a history of pre-eclampsia had carotid plaque present on ultrasound.

Table 5.8 Results of vascular studies for all 166 COPS study participants

	Normotensive Controls (N=80)	**Pre-eclampsia Cases (N=86)**	**P-value**
Mean AIx @ HR 75bpm (%)	22.5±9.6	25.7±11	**0.023**
Mean aortic SBP (mmHg)	110±11	118±16	**<0.001**
Mean aortic DBP (mmHg)	75±8	78±9	**0.007**
Mean arterial pressure (MAP)	89.9±8.5	95.4±11.1	**<0.001**
Mean cfPWV (m/s)	7.1±1.1	7.8±1.6	**0.001**
Mean cIMT CCA (mm)	0.622±0.112	0.655±0.132	0.088
Mean cIMT BULB (mm)	0.679±0.146	0.676±0.148	0.807
Mean cIMT ICA (mm)	0.553±0.135	0.552±0.125	0.961
Plaque count	25/79 (31.6%)	43/84 (51.2%)	**0.011**
Plaque score	0.384±0.603	0.733±0.852	**0.006**
Baseline brachial artery diameter (mm)	3.62±0.45	3.65±0.49	0.686
Maximum brachial artery diameter (mm)	3.87±0.47	3.86±0.49	0.918
%FMD	7.01±3.31	5.93±3.29	**0.016**

AIx@HR75 – augmentation index (here it is adjusted for heart rate), HR – heart rate, SBP – systolic blood pressure, DBP – diastolic blood pressure, cfPWV – carotid-femoral pulse wave velocity, cIMT – carotid intima-media thickness, CCA – common carotid artery, ICA – internal carotid artery, FMD – flow-mediated dilatation. Data are presented as mean ± SD for continuous data or as a proportion for categorical data. For AIx@HR75, aortic blood pressures and cfPWV, "mean" is the mean of 2 readings recorded at the study visit. For cIMT measurements "mean" is the mean of the value from the right and left sides.

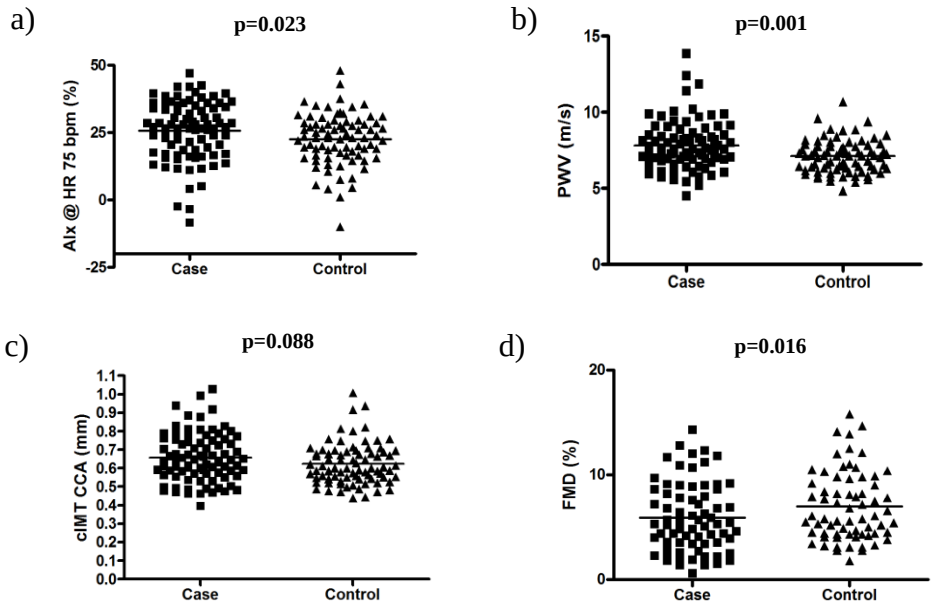

Figure 5.8 Results of COPS vascular studies

a) Mean heart-rate adjusted augmentation index (AIx@HR75) on pulse wave analysis measured as percent b) Mean carotid-femoral pulse wave velocity in metres/second (PWV) c) carotid intima-media thickness at common carotid artery in millimetres (cIMT) d) flow-mediated dilatation (FMD) in pre-eclampsia measured as a percent. All data are grouped by pre-eclampsia case or control status.

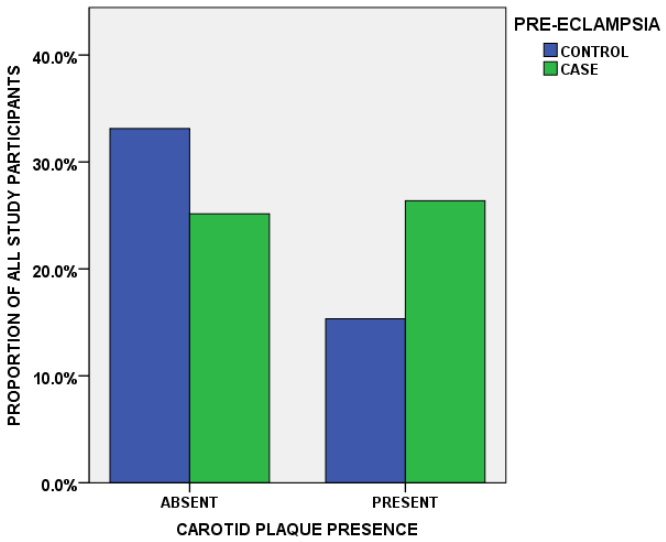

Figure 5.9 Bar chart of carotid plaque presence

Pre-eclampsia cases are coloured green and normotensive controls are in blue.

For all subsequent analyses Pearson's or Spearman's correlation were used as appropriate and for regression the data fulfilled the assumptions of multiple regression analysis.

5.3.3.1 *Heart-rate adjusted augmentation index*

Heart-rate adjusted augmentation index (AIx@HR75) was not significantly correlated with weight, BMI, resting heart rate, or HDL. It did correlate significantly with age (ρ=0.531, p=<0.001), height (ρ= -0.350, p=<0.001), SBP (ρ=0.450, p=<0.001), DBP (ρ=0.340, p=<0.001), MAP (ρ=0.570, p=<0.001) total cholesterol (ρ=0.238, p=0.002), non-HDL cholesterol (ρ=0.283, p=<0.001) and triglycerides (ρ=0.285, p=<0.001). It did not correlate with age at index pregnancy, gestation at index pregnancy, birth weight or birth weight z-score, but it was significantly correlated with years since index pregnancy (ρ=0.509, p=<0.001). See figures 5.10 and 5.11 for scatterplots.

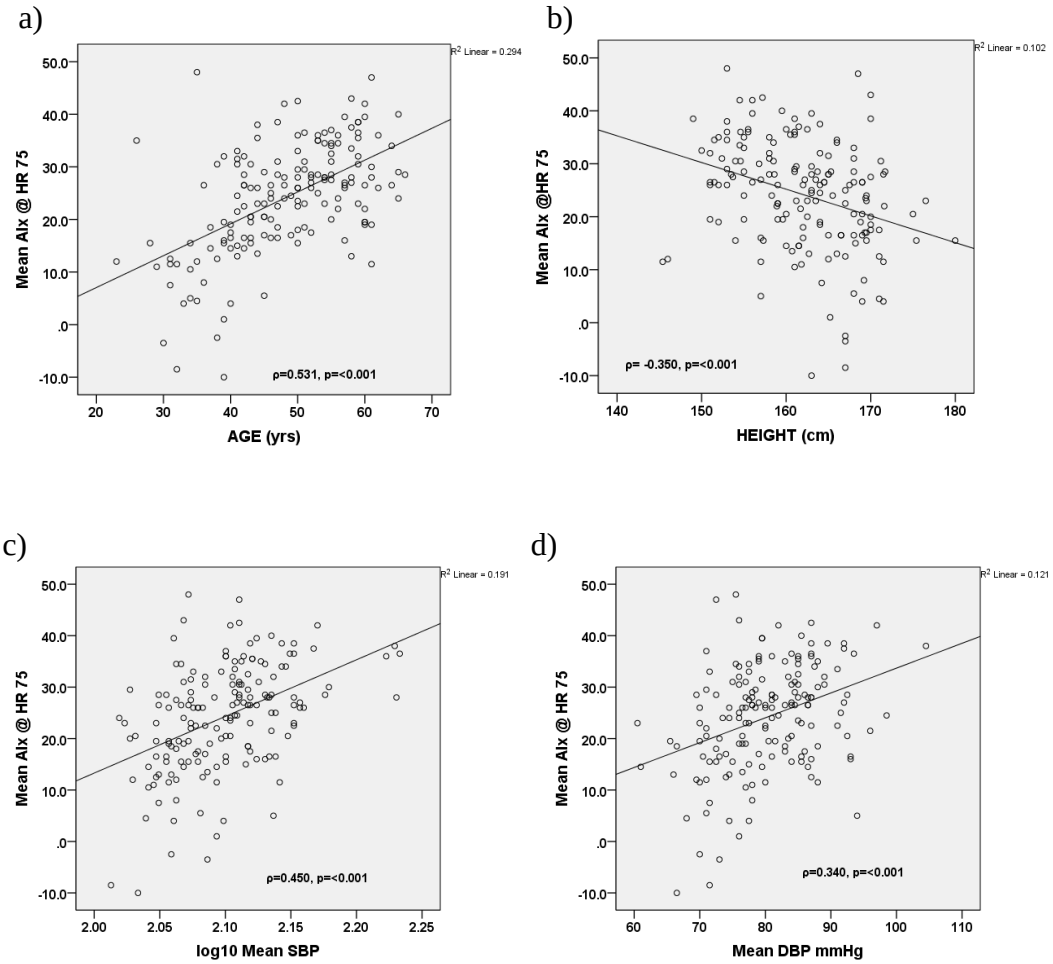

Figure 5.10 Scatterplots of age, height, systolic blood pressure and diastolic blood pressure against mean heart-rate adjusted augmentation index

a) scatterplot of age vs mean AIx@HR75, b) scatterplot of height vs mean AIx@HR75, c) scatterplot of SBP vs AIx@HR75, b) scatterplot of DBP vs AIx@HR75.

AIx@HR75 = heart-rate adjusted augmentation index, SBP = systolic blood pressure in mmHg, DBP = diastolic blood pressure in mmHg.

Spearman's correlation coefficients (rho) have been calculated. Regression lines have been added only to indicate direction of the relationship.

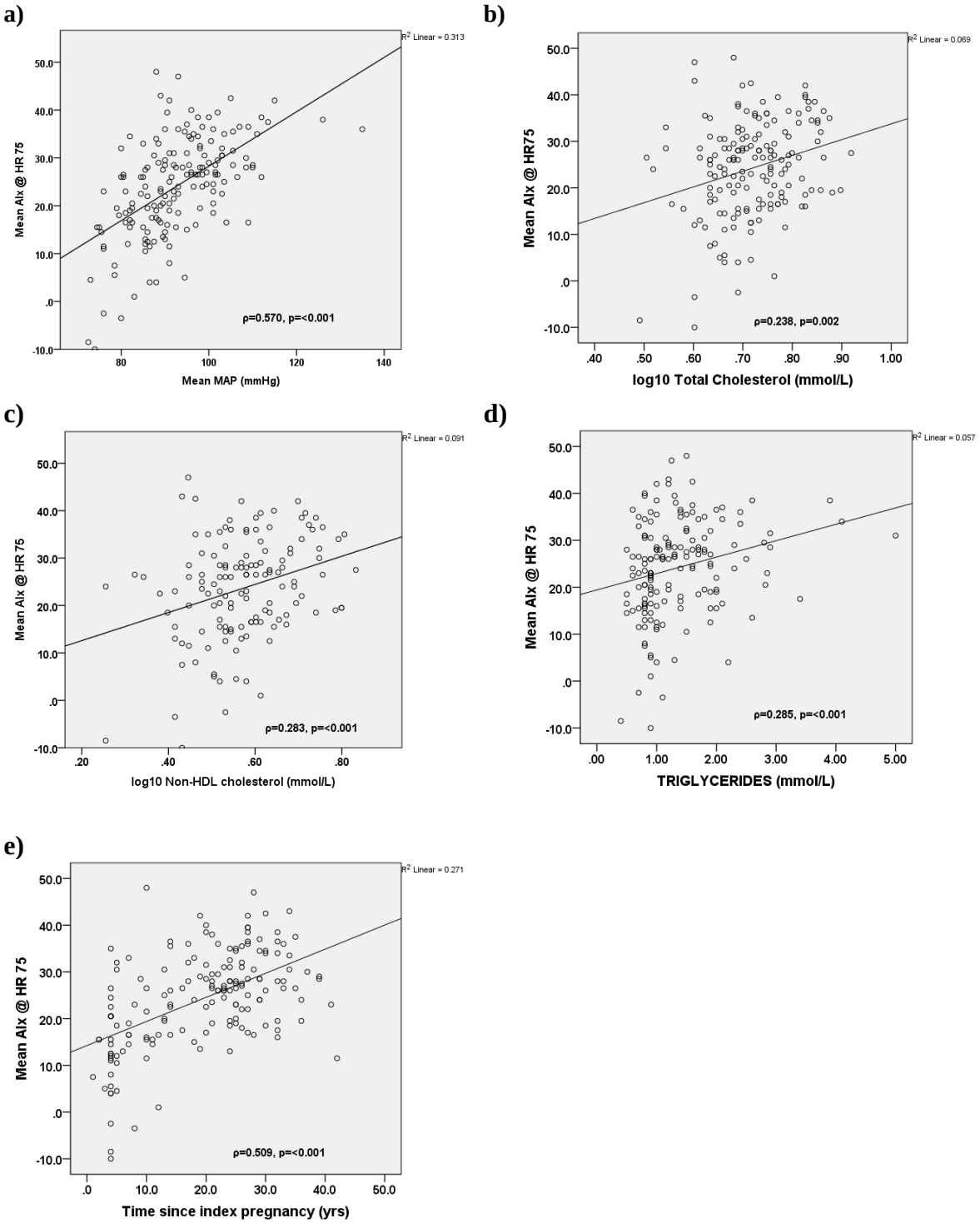

Figure 5.11 Scatterplots of other variables against heart-rate adjusted augmentation index

a) scatterplot of MAP vs AIx@HR75, b) scatterplot of total cholesterol vs AIx@HR75, c) scatterplot of non-HDL cholesterol vs AIx@HR75, d) scatterplot of triglycerides vs AIx@HR75 e) scatterplot of years since index pregnancy vs AIx@HR75. AIx@HR75 = heart-rate adjusted augmentation index, MAP = mean arterial pressure, HDL = high-density lipoprotein.

Spearman's correlation coefficients (rho) have been calculated. Regression lines have been added only to indicate direction of the relationship.

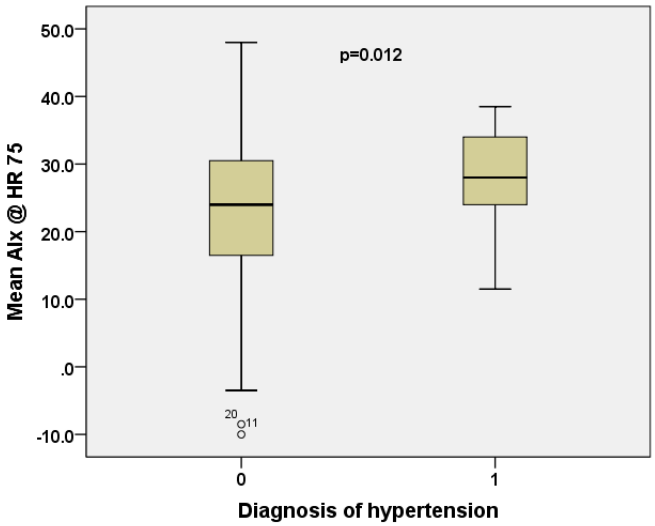

Figure 5.12 Boxplot of AIx@HR75 by diagnosis of hypertension

There was a significant difference in AIx@HR75 when separated by women with a diagnosis of hypertension and those without, on independent samples t-test (p=0.012) (Figure 5.12), but there was no difference in smoking status.

Age, height, SBP, DBP, MAP, cholesterol, non-HDL cholesterol, triglycerides, time since pregnancy and diagnosis of hypertension were entered into a multiple regression model as independent variables, along with history of pre-eclampsia. AIx@HR75 was the dependent variable. History of pre-eclampsia was not a statistically significant determinant of AIx@HR75 (p=0.472). Therefore, history of pre-eclampsia is not an independent determinant of AIx@HR75 later in life. Age, height and MAP were significant determinants of AIx@HR75. The same was seen when a more comprehensive model which included other cardiovascular risk factors was used. In this model age, BMI, SBP, cholesterol, diabetes, smoking and pre-eclampsia status were entered as independent variables. Pre-eclampsia p-value=0.146 indicating it was not a determinant of AIx@HR75. Age and SBP were significant determinants of AIx@HR75 (both p-values <0.001).

5.3.3.2 *Carotid-femoral pulse wave velocity*

Carotid-femoral pulse wave velocity (cfPWV) was not significantly correlated with height, resting heart rate, total cholesterol or birth weight z-score. However it was significantly correlated with age (r=0.420, p=<0.001), weight (r=0.272, p=0.001), BMI (ρ=0.353, p=<0.001), SBP (r=0.503, p=<0.001), DBP (r=0.327, p=<0.001), MAP (r=0.522, p=<0.001), non-HDL cholesterol (r=0.190, p=0.029) and triglycerides (ρ=0.247, p=0.002) (Figure 5.13).

Age at index pregnancy was significantly negatively correlated with cfPWV (ρ= - 0.218, p=0.005) and years since index pregnancy was significantly positively correlated (ρ= 0.453, p=<0.001) (Figure 5.14). There was a significant difference in cfPWV between hypertensive cases and controls on independent samples t-test (p=<0.001) (Figure 5.15), but there was no difference in smoking status.

On multiple regression analysis, which included all of the above significant variables and pre-eclampsia, a history of pre-eclampsia was not statistically significant at p=0.978. Only MAP was significant in determining cfPWV with p=0.012. Pre-eclampsia was therefore not an independent determinant of cfPWV and on entering a model of pre-eclampsia with classical cardiovascular risk factors it was still not significant in determining cfPWV later in life (p=0.153). In this model age, SBP and BMI were all significant with p=<0.001 for each of them.

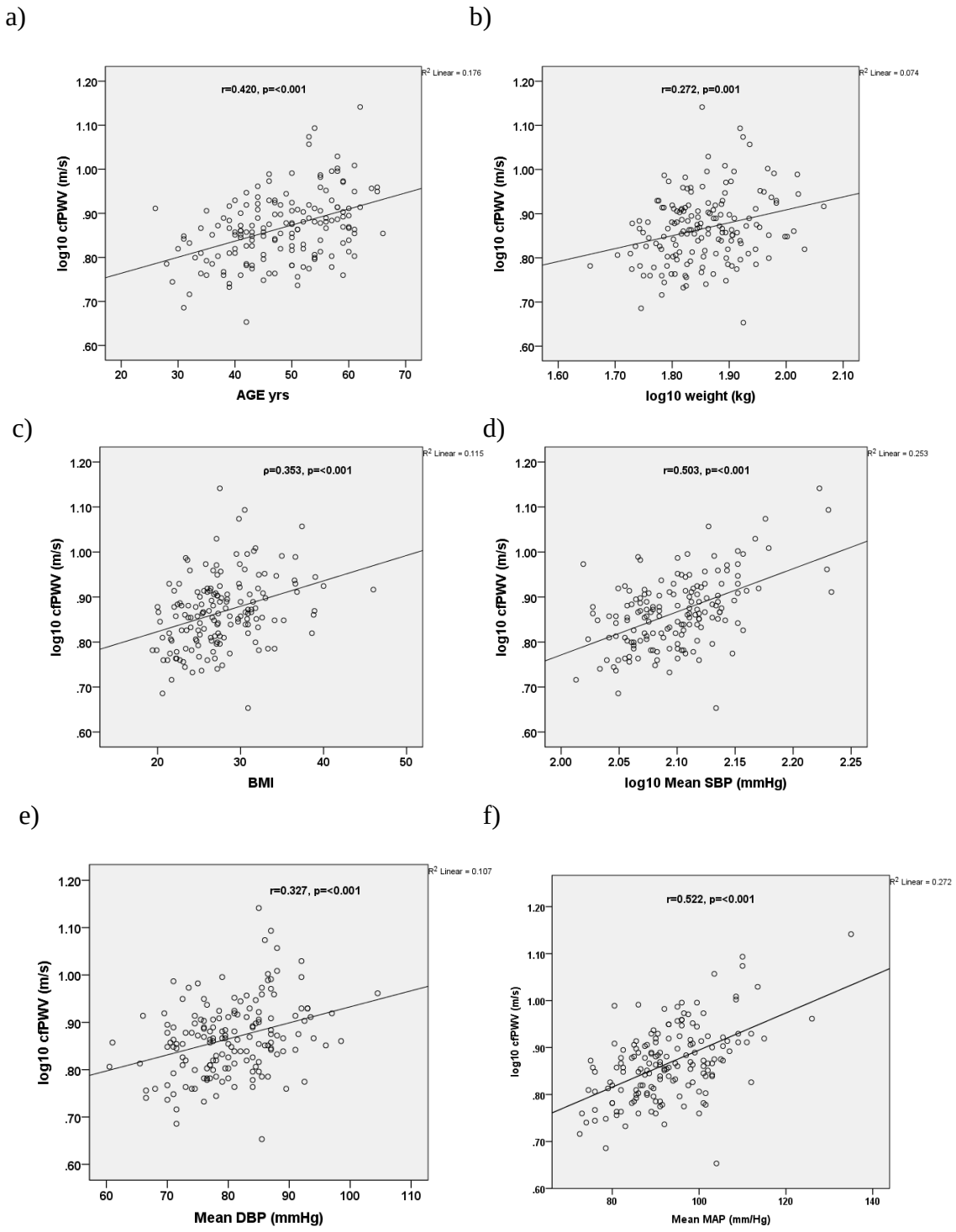

Figure 5.13 Scatterplot 1 of variables against carotid femoral pulse wave velocity

a) scatterplot of age vs log cfPWV b) scatterplot of weight vs log cfPWV c) scatterplot of BMI vs log cfPWV d) scatterplot of SBP vs log cfPWV e) scatterplot of DBP vs log cfPWV f) scatterplot of MAP vs log cfPWV . CfPWV = carotid-femoral pulse wave velocity, BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, MAP = mean arterial pressure.

Where Spearman's correlation coefficients (rho) have been calculated, regression lines have been added only to indicate direction of the relationship.

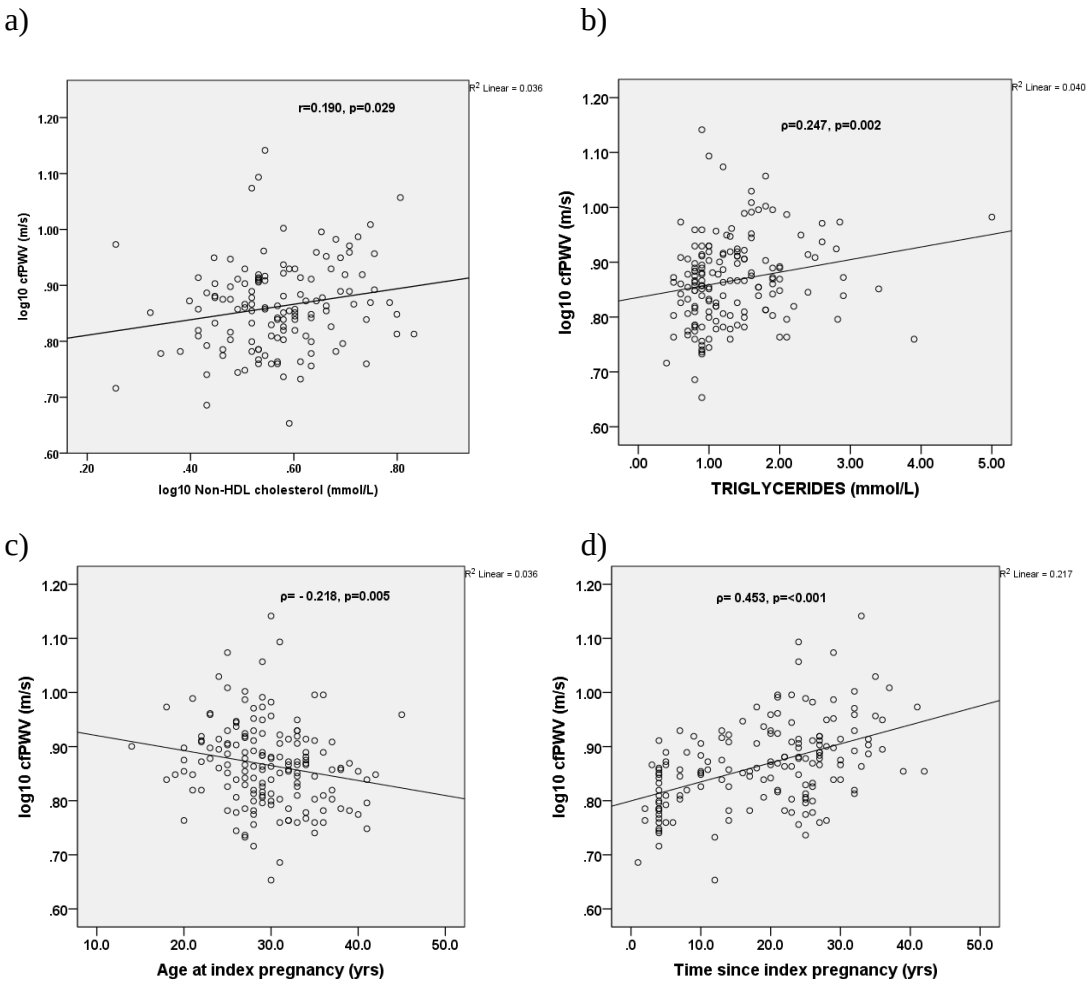

Figure 5.14 Scatterplot 2 of variables against carotid femoral pulse wave velocity

a) scatterplot of non-HDL cholesterol vs log cfPWV b) scatterplot of triglycerides vs log cfPWV, c) scatterplot of age at index pregnancy in yrs vs log cfPWV, d) scatterplot of time since index pregnancy vs log cfPWV. CfPWV = carotid-femoral pulse wave velocity, HDL = high-density lipoprotein.

Spearman's correlation coefficients (rho) have been calculated. Regression lines have been added only to indicate direction of the relationship.

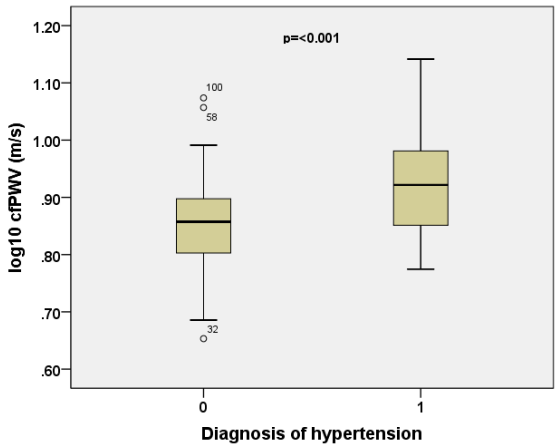

Figure 5.15 Boxplot of log cfPWV by hypertension status

5.3.3.3 *Flow-mediated dilatation*

Flow-mediated dilatation was only significantly negatively correlated with weight (r= -0.186, p=0.029) (Figure 5.16). There was no difference with hypertension status or smoking status on independent samples t-test.

On multiple regression, for the first model, pre-eclampsia was entered with weight. Pre-eclampsia was significant (p=0.041) in determining FMD. Weight was no longer significant. Following this, pre-eclampsia was entered into a model with, age, BMI and SBP and it was found to be statistically significant in determining FMD (p=0.022). None of the other variables were significant. As expected there was an inverse correlation between baseline brachial artery diameter and flow-mediated dilatation (r=-0.351, p<0.001). However, history of pre-eclampsia remained a significant determinant of FMD even if baseline brachial artery diameter was entered into the above regression models (data not shown).

Finally, on entering pre-eclampsia into a model with classical cardiovascular risk factors (age, BMI, SBP, total cholesterol, diabetes, smoking status) the overall model was not significant (F=1.633, p=0.132). However pre-eclampsia remained significant in determining FMD after adjusting for all other variables. (p=0.040). No other variable in the model was significant in determining FMD.

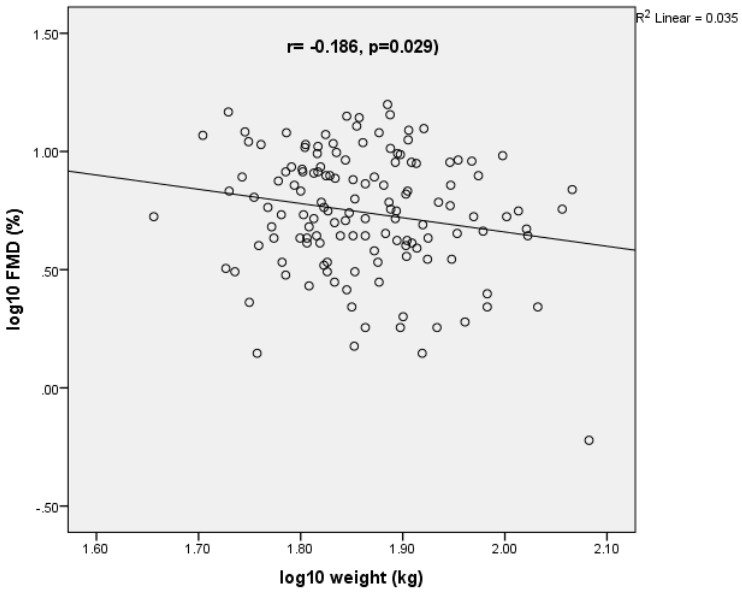

Figure 5.16 Scatterplot of weight against flow-mediated dilatation

5.3.3.4 *Carotid plaque score*

Plaque score did not correlate with age, blood pressure, MAP, cholesterol, non-HDL cholesterol, triglycerides or high-density lipoprotein. It did correlate with BMI (ρ=0.161, p=0.041) and AIx @HR75 (ρ=0.174, p=0.026) but not with PWV or FMD. Mean cIMT at the common carotid artery and mean cIMT at the carotid bulb were correlated with plaque score (ρ= 0.229, p= 0.003 and ρ=0.244, p=0.002 respectively) but not mean cIMT at the internal carotid artery.

Entering the significant variables along with pre-eclampsia into a binary logistic regression model for the presence or absence of carotid plaque, pre-eclampsia was a determinant of presence of carotid plaque (p=0.019). On entering the cardiovascular model of age, BMI, SBP, total cholesterol, diabetes, and smoking status, pre-eclampsia remained significant in determining plaque presence after adjusting for the other cardiovascular risk factors (p=0.018). None of the other variables was significant in determining the presence of carotid plaques.

5.3.4 **Further subgroup analysis**

Previous studies assessing vascular function after hypertensive disorders of pregnancy have found differences according to age of participants at time of recruitment (whether older or younger) and depending on length of time since index pregnancy (142;143;395;400-402). In the meta-analysis described above (179) the differences in vascular function were found to be more notable in younger subjects aged <40years or subjects closer to the time of index pregnancy. While adjustments have been made for a variety of co-variates on performing regression analysis (section 5.3.3), further subgroup analysis was performed, to explore any obvious differences depending on grouping for age (Tables 5.9-5.10) or for time since index pregnancy (Tables 5.11-5.12).

Women <50 years at time of recruitment to the COPS study showed significantly higher BMI, lower age at time of index pregnancy, lower gestation at delivery and lower birth weight in the pre-eclampsia group than the normotensive control group. There were no significant differences in blood pressure or vascular function studies in women under 50 years (Table 5.9). In comparison, women age ≥ 50 years at time of recruitment followed the same pattern, for BMI, gestation at delivery and birth weight, but in addition women with a history of pre-eclampsia also had significant differences in baseline blood pressure readings, central blood pressure, adjusted AIx, cfPWV, cIMT at the common carotid artery and flow-mediated dilatation. Women with history of pre-eclampsia also had a higher proportion requiring C-section (Table 5.10).

Table 5.9 Results for women aged <50years at time of COPS study visit

	Normotensive Controls (N=38)	**Pre-eclampsia Cases (N=46)**	**P-value**
Age at study visit (yrs)	41.2±4.5	39.9±6.6	0.620
Height (cm)	163.5±6.7	162.0±7.1	0.344
Weight (kg)	72.3±13.0	77.9±15.9	0.087
BMI (kg/m^2)	27.1±5.0	29.8±6.5	**0.041**
Mean* SBP sitting (mmHg)	122±10	126±13	0.129
Mean* DBP sitting (mmHg)	79±3	82±9	0.061
Resting HR (bpm)	72±10	75±11	0.145
SBP supine (mmHg)	118±11	123±14	0.068
DBP supine (mmHg)	74±9	76±11	0.334
Mean AIx at HR 75bpm	18.6±10.6	20.0±11.0	0.553
Mean aortic SBP (mmHg)	108±12	112±15	0.134
Mean aortic DBP (mmHg)	75±9	77±11	0.186
Total cholesterol (mmol/L)	5.0±0.7	4.9±0.9	0.156
Triglycerides (mmol/L)	1.3±0.6	1.2±0.6	0.180
HDL	1.5±0.3	1.4±0.3	0.678
Mean cfPWV (m/s)	6.9±1.0	7.2±1.1	0.186
Mean cIMT CCA (mm)	0.587±0.09	0.584±0.09	0.942
Mean cIMT BULB (mm)	0.621±0.109	0.623±0.135	0.890
Mean cIMT ICA (mm)	0.518±0.126	0.510±0.117	0.766
Plaque score	0.287±0.493	0.596±0.795	0.076
Plaque presence	10/38 (26.3%)	19/45 (42.2%)	0.130
FMD baseline (mm)	3.58±0.46	3.56±0.49	0.769
FMD maximum (mm)	3.84±0.46	3.78±0.51	0.590
%FMD	7.15±3.31	6.23±3.09	0.240
Age at index pregnancy (yrs)	31.0±6.2	28.2±5.4	**0.027**
Yrs since index pregnancy at time of study visit (yrs)	10.6±7.7	12.3±8.3	0.432
Gestation at delivery (wks)	39.6±1.9	37.3±3.8	**<0.001**
Birthweight (g)	3359±605	2981±754	**0.017**
C-section	14/38 (36.8%)	16/46 (34.8%)	0.845

BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, HDL = high density lipoprotein. AIx – augmentation index (here it is adjusted for heart rate), HR – heart rate, cfPWV – carotid-femoral pulse wave velocity, cIMT – carotid intima-media thickness, CCA – common carotid artery, ICA – internal carotid artery, FMD – flow-mediated dilatation. Data are presented as mean ± SD for continuous data or as a proportion for categorical data.

*** Mean of the 2nd and 3rd blood pressure readings taken.**

Table 5.10 Results for women aged ≥50years at time of COPS study visit

	Normotensive Controls (N=42)	**Pre-eclampsia Cases (N=40)**	**P-value**
Age at study visit (yrs)	55.7±4.5	56.6±4.2	0.390
Height (cm)	162.0±6.7	160.7±5.8	0.337
Weight (kg)	68.2±8.8	74.7±14.0	0.054
BMI (kg/m^2)	26.1±3.9	29.0±5.7	**0.020**
Mean* SBP sitting (mmHg)	123±10	134±14	**<0.001**
Mean* DBP sitting (mmHg)	77±7	83±6	**<0.001**
Resting HR (bpm)	70±8	71±11	0.701
SBP supine (mmHg)	119±10	130±16	**<0.001**
DBP supine (mmHg)	74±7	79±8	**0.002**
Mean AIx at HR 75bpm	26.1±7.0	32.2±6.5	**<0.001**
Mean aortic SBP (mmHg)	113±10	124±15	**<0.001**
Mean aortic DBP (mmHg)	75±7	80±8	**0.001**
Total cholesterol (mmol/L)	5.7±1.1	5.8±1.0	0.718
Triglycerides (mmol/L)	1.4±0.8	1.6±0.8	0.260
HDL	1.6±0.4	1.6±0.4	0.968
Mean cfPWV (m/s)	7.3±1.1	8.6±1.8	**<0.001**
Mean cIMT CCA (mm)	0.655±0.118	0.738±0.124	**0.003**
Mean cIMT BULB (mm)	0.733±0.157	0.738±0.140	0.881
Mean cIMT ICA (mm)	0.585±0.137	0.602±0.118	0.463
Plaque score	0.473±0.684	0.892±0.897	**0.025**
Plaque presence	15/41 (36.6%)	24/39 (61.5%)	**0.026**
FMD baseline (mm)	3.65±0.46	3.76±0.48	0.301
FMD maximum (mm)	3.90±0.48	3.96±0.45	0.537
%FMD	6.89±3.33	5.55±3.53	**0.031**
Age at index pregnancy (yrs)	29±5	30±5	0.628
Yrs since index pregnancy at time of study visit (yrs)	27.1±6.6	27.1±5.4	0.577
Gestation at delivery (wks)	39.7±2.3	38.0±2.5	**<0.001**
Birthweight (g)	3431±457	3013±681	**0.002**
C-section	5/42 (11.9%)	13/40 (32.5%)	**0.024**

BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, HDL = high density lipoprotein. AIx – augmentation index (here it is adjusted for heart rate), HR – heart rate, cfPWV – carotid-femoral pulse wave velocity, cIMT – carotid intima-media thickness, CCA – common carotid artery, ICA – internal carotid artery, FMD – flow-mediated dilatation. Data are presented as mean ± SD for continuous data or as a proportion for categorical data.

*** Mean of the 2nd and 3rd blood pressure readings taken.**

On subgroup analysis evaluating length of time since index pregnancy, findings were similarly different, with more significant results in pregnancies which had occurred longer ago.

Women with more recent index pregnancies ≤20 years ago showed significant differences in the areas of age at pregnancy (with pre-eclampsia cases being younger at time of delivery), gestation at delivery and birthweight (both of these values being lower in the pre-eclampsia group). There were no other significant differences between cases and normotensive controls (see Table 5.11), in particular, there were no differences in vascular study results.

In contrast, when index pregnancies >20 years ago were analysed, pre-eclampsia cases had significantly higher BMI, SBP, DBP, significantly lower gestational age and birthweight at delivery and a higher proportion with C-section. Vascular studies revealed significantly higher adjusted AIx, higher central SBP and DBP, higher cfPWV, higher cIMT CCA and lower FMD % in the pre-eclampsia group than the controls (see Table 5.12).

Table 5.11 Results for women at ≤ 20 years since index pregnancy

	Normotensive Controls (N=34)	**Pre-eclampsia Cases (N=44)**	**P-value**
Age at study visit (yrs)	41±5	41±9	0.764
Height (cm)	163.8±6.7	161.7±6.8	0.173
Weight (kg)	72.0±12.2	76.5±16.1	0.180
BMI (kg/m^2)	26.9±4.6	29.4±6.7	0.077
Mean* SBP sitting (mmHg)	122±10	125±11	0.266
Mean* DBP sitting (mmHg)	79±8	82±8	0.077
Resting HR (bpm)	71±10	73±10	0.239
SBP supine (mmHg)	117±11	122±12	0.087
DBP supine (mmHg)	73±8	75±9	0.338
Mean AIx at HR 75bpm	17.9±11.1	20.5±11.7	0.331
Mean aortic SBP (mmHg)	107±12	112±12	0.109
Mean aortic DBP (mmHg)	74±9	77±10	0.177
Total cholesterol (mmol/L)	5.0±0.6	4.8±0.8	0.271
Triglycerides (mmol/L)	1.3±0.6	1.1±0.6	0.100
HDL	1.5±0.3	1.5±0.3	0.570
Mean cfPWV (m/s)	6.8±1.0	7.0±1.0	0.372
Mean cIMT CCA (mm)	0.590±0.100	0.604±0.113	0.617
Mean cIMT BULB (mm)	0.634±0.124	0.624±0.132	0.644
Mean cIMT ICA (mm)	0.509±0.107	0.514±0.103	0.848
Plaque score	0.365±0.612	0.586±0.735	0.191
Plaque presence	10/34 (29.4%)	19/43 (44.2%)	0.184
FMD baseline (mm)	3.64±0.46	3.55±0.50	0.386
FMD maximum (mm)	3.89±0.47	3.77±0.52	0.292
%FMD	6.84±3.03	6.29±3.09	0.481
Age at index pregnancy (yrs)	33.1±4.7	30.4±5.5	**0.021**
Yrs since index pregnancy at time of study visit (yrs)	8.1±4.9	10.7±6.4	0.123
Gestation at delivery (wks)	39.4±2.0	37.2±3.6	**<0.001**
Birthweight (g)	3326±613	2889±793	**0.011**
C-section	15/34 (44.1%)	17/44 (38.6%)	0.626

BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, HDL = high density lipoprotein. AIx – augmentation index (here it is adjusted for heart rate), HR – heart rate, cfPWV – carotid-femoral pulse wave velocity, cIMT – carotid intima-media thickness, CCA – common carotid artery, ICA – internal carotid artery, FMD – flow-mediated dilatation. Data are presented as mean ± SD for continuous data or as a proportion for categorical data.

*** Mean of the 2nd and 3rd blood pressure readings taken.**

Table 5.12 Results for women >20 years since index pregnancy

	Normotensive Controls (N=46)	**Pre-eclampsia Cases (N=42)**	**P-value**
Age at study visit (yrs)	54.7±5.5	55.1±5.2	0.715
Height (cm)	161.9±6.6	161.1±6.2	0.545
Weight (kg)	68.8±10.3	76.3±14.1	**0.004**
BMI (kg/m^2)	26.3±4.4	29.5±5.5	**0.007**
Mean* SBP sitting (mmHg)	123±10	135±15	**<0.001**
Mean* DBP sitting (mmHg)	78±7	84±8	**<0.001**
Resting HR (bpm)	71±8	72±11	0.534
SBP supine (mmHg)	119±9.7	131±17	**<0.001**
DBP supine (mmHg)	74±7	79±9	**0.004**
Mean AIx at HR 75bpm	25.9±6.6	31.1±7.0	**0.001**
Mean aortic SBP (mmHg)	112±11	124±17	**<0.001**
Mean aortic DBP (mmHg)	75±7	80±9	**0.002**
Total cholesterol (mmol/L)	5.6±1.1	5.8±1.0	0.563
Triglycerides (mmol/L)	1.4±0.8	1.6±0.8	0.135
HDL	1.5±0.4	1.5±0.4	0.844
Mean cfPWV (m/s)	7.3±1.1	8.7±1.7	**<0.001**
Mean cIMT CCA (mm)	0.647±0.117	0.709±0.131	**0.022**
Mean cIMT BULB (mm)	0.713±0.154	0.731±0.144	0.568
Mean cIMT ICA (mm)	0.588±0.146	0.593±0.135	0.839
Plaque score	0.398±0.603	0.888±0.943	**0.008**
Plaque presence	15/45 (33.3%)	24/41 (58.5%)	**0.019**
FMD baseline (mm)	3.61±0.46	3.77±0.47	0.137
FMD maximum (mm)	3.86±0.48	3.97±0.43	0.311
%FMD	7.11±3.51	5.52±3.50	**0.018**
Age at index pregnancy (yrs)	27.7±4.9	27.5±4.5	0.715
Yrs since index pregnancy at time of study visit (yrs)	27.4±5.6	28.0±4.1	0.147
Gestation at delivery (wks)	39.8±2.2	38.2±2.8	**<0.001**
Birthweight (g)	3450±461	3108±615	**0.005**
C-section	4/46 (8.7%)	12/42 (28.6%)	**0.016**

BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, HDL = high density lipoprotein. AIx – augmentation index (here it is adjusted for heart rate), HR – heart rate, cfPWV – carotid-femoral pulse wave velocity, cIMT – carotid intima-media thickness, CCA – common carotid artery, ICA – internal carotid artery, FMD – flow-mediated dilatation. Data are presented as mean ± SD for continuous data or as a proportion for categorical data.

*** Mean of the 2nd and 3rd blood pressure readings taken.**

5.4 **Discussion**

5.4.1 **Findings**

The key findings from this study were: 1) blood pressure was higher later in life in women with a history of pre-eclampsia, 2) gestational age at delivery and birth weight were lower in comparison with normotensive controls, however birth weight was not significantly different after adjusting for gestational age, 3) AIx @HR75, cfPWV and FMD were all significantly different between cases and controls but only FMD was determined by pre-eclampsia status when adjusted for relevant confounders, and 4) women with a history of pre-eclampsia had a higher plaque score.

This impaired FMD was not accounted for by other cardiovascular risk factors and it would appear that pre-eclampsia was an independent risk factor for impaired FMD in this study. Decreased FMD in women with a more remote history of pre-eclampsia in this study is different to the findings of other studies examining endothelial function after a pre-eclamptic pregnancy. FMD has been found to be significantly lower in cases with pre-eclampsia, closer to the time of the index pregnancy (136;138;139) and normalising after a decade (142;143). However, it is not surprising that FMD, a measure of endothelial function, would be the first modality to show evidence of change as endothelial function would be expected to be the earliest evidence of cardiovascular disease, as it precedes more structural alterations of cardiovascular disease in the blood vessels such as atherosclerosis. Henriques et al (402) found evidence of decreased FMD at 15 years following a pre-eclamptic pregnancy. On review of the literature this appears to be the only other study to date which shows any evidence of endothelial dysfunction after ~10 years when analysed by FMD. Regarding the women in whom there has been normalisation of endothelial dysfunction at 10 years after pre-eclampsia, it is not possible to know whether they would have started to show any evidence of endothelial dysfunction if they had been examined again at 15 years. Likewise, it is not possible to know what the vascular findings of COPS study subjects would have been 10 or 15 years ago. In this respect, the value of long-term prospective follow-up studies is considerable.

Another source of potential difference between studies is the study protocol ranging from number of patients recruited, preparation of patient (fasting vs non-fasting), timing of the

study visit (morning vs afternoon), number of operators, differences in FMD equipment and analysis software to name a few. There are many confounders which must be taken into account when comparing studies.

In the COPS study, age was not a predictor of FMD and it was important to establish whether or not age was driving the difference observed between cases and controls. There is evidence that after the age of 50yrs, FMD will decrease by 0.49% per year in women (403). Of note is also the seemingly low value for FMD in the COPS study population in general, even among the controls, in comparison with other studies in the field. However, in a study of age-related changes in vascular function (404), 56 healthy women aged 35 years had FMD 5.7±3.5% and 47 women aged 55 years had FMD 2.6±2.3% (one woman in this group had suffered previous myocardial infarction and four women were on antihypertensive medication). These values, even in young healthy 35 year olds are lower than those found in the COPS study. Overall, the COPS FMD values in the control population were more consistent with the values for women in a study which specifically aimed to establish reference values for FMD (405), which lends confidence to the conclusions reached in the COPS study.

cIMT values were not different between groups in the COPS vascular study and there are conflicting reports in the literature regarding change in cIMT. There is evidence that pre-eclampsia does not significantly increase carotid intima-media thickness (143). However there are a few studies which have shown some evidence of an increased cIMT. A study by Goynumer et al reported a significantly higher cIMT an average of 19 months after index pregnancy (138). Akhter et al (406) calculated the individual intima and individual media layers of the common carotid artery (CCA) and found that in women with severe pre-eclampsia there was a significantly thicker CCA intima and intima/media ratio but the intima-media thickness itself was not significantly different between groups. Aykas et al (407) found increased cIMT at 5 years after index pregnancy. In the COPS study carotid plaque presence and plaque score were significantly higher in the pre-eclampsia group than controls and carotid plaque presence has been found to be an important predictor of cardiovascular risk in women (196).

How might the difference in carotid plaques be explained against the absence of a difference in cIMT? First it is important to understand how different risk factors might influence cIMT and carotid plaque presence. In a recent study of 553 patients (337 women and 216 men) which sought to assess the different effects of traditional cardiovascular risk

factors on cIMT and plaque presence (408), the authors found that the probability of plaque occurrence was higher in patients with coronary artery disease and dyslipidaemia and was influenced by age but not hypertension or diabetes. Hypertension most increased cIMT, and when associated with coronary artery disease, diabetes and dyslipidaemia. cIMT measurements were significantly increased in the presence of hypertension, while blood lipid levels were more associated with carotid plaque formation (408;409). Hypertension was not found to contribute to the presence of plaque. In the COPS study on subgroup analysis of older women, there was a significantly higher cIMT along with a significant increase in blood pressure which would be in keeping with the literature. While there was a significantly higher plaque score, there was no difference in cholesterol level. There could be other confounders associated with the presence of carotid plaque (such as socioeconomic status) which were outwith the scope of this study, and further research is warranted.

5.4.2 **Strengths**

Sample size for the COPS vascular study exceeds many previous studies in the field (139-142;178;400;402;407;410-415). The benefits of a larger sample size include decreasing the likelihood of a false result. This is particularly important when the findings of a study contradict a substantial proportion of other published work in the field.

There were a wide range of vascular studies performed in COPS, with thorough consideration as to how to detect any evidence of early subclinical vascular damage. In a recent meta-analysis of vascular dysfunction (179), none of the 37 studies included had evaluated as many different modalities for assessing vascular function (in one study visit) as COPS and this is a particular strength.

The age range of recruitment meant further exploration of the data was possible such as how parameters varied depending on age or time since index pregnancy. For example, younger women were less likely to have differences in vascular function, and such differences were more exaggerated in women further from the time of index pregnancy.

Another strength of this study is that it focussed on women at longer time-points since the index pregnancy than most other studies. The range for inclusion was up to 30yrs after pregnancy and average time since index pregnancy was ~20years. It is possible that vascular changes and evidence of endothelial dysfunction after a pregnancy complicated

by pre-eclampsia may vary over time, and while most other studies have continued for up to 10-11 years after the index pregnancy (142;143), relatively few have explored vascular function beyond this time (400). There have been reports in the literature of normalisation of endothelial dysfunction (which was present in the postpartum period) by 10 years after delivery (142;143).

5.4.3 **Limitations**

In the COPS study women were non-fasted, attended in the morning or the afternoon (timing of study visit was noted), were at varying stages throughout the menstrual cycle or postmenopausal and were inconsistencies regarding medication (e.g. hormonal contraception, hormone replacement therapy, antihypertensive therapies). These differences were not corrected for on analysis.

Another weakness of the COPS study was that not all maternity records were reviewed. Some previous studies have used questionnaires and self-reported history of pregnancy in ascertainment of hypertension in pregnancy or pre-eclampsia in pregnancy. One such study at the Mayo Clinic, Minnesota (416) sought to validate a pre-eclampsia questionnaire and, when compared with Maternity records, they verified a diagnosis of pre-eclampsia, eclampsia or toxaemia with 80% sensitivity and 96% specificity. The study was published in 2008 and was based on pregnancies between 1960-1979, so all participant recall was for events more than 20 years previously (416). However, there is always a potential for differing terminology over time affecting the recall diagnosis in more remote pregnancies. Nevertheless, maternity records which were checked in the COPS study showed excellent concordance with the study questionnaire data. Moreover, hypothetically, if any women had been misclassified and had been allocated to the wrong group, a detectable difference in the vascular study between groups would have been less likely, not more likely.

For the COPS vascular study there were two operators, and while we were both equally as skilled and trained in performing vascular studies, this could potentially have introduced bias. The literature suggests very good reproducibility and repeatability for heart-rate adjusted augmentation index (AIx) and carotid-femoral pulse wave velocity (cfPWV) (417;418). Carotid intima-media thickness (cIMT) and flow-mediated dilatation (FMD) have also been found in previous studies to be reproducible (116;118;419-421). However, cIMT and FMD are considered to be more operator-dependent than other methods, and in view of this we trained extensively together, to acquire the same level of expertise. Intra-

operator and inter-operator assessments were performed with excellent agreement as outlined in section 5.2.6.7.

Sub-group analysis according to severity of pre-eclampsia, as assessed for example by gestational age at delivery or by the presence of eclampsia or HELLP syndrome was not performed. While this would have been possible, given the information available, any results would have been likely to have been underpowered due to small sample size.

The index pregnancy was defined as in section 5.2.3. This was not necessarily the first pregnancy for controls, if they happened to experience a miscarriage initially, nor was it necessarily the first pregnancy in the pre-eclampsia group. Miscarriages have been found to confer an increased risk of cardiovascular disease (383;422). The fact that there is inconsistency between women as to which actual chronological pregnancy was being used is something which was not corrected for, and it would not be possible to exclude some level of bias or confounding as a result. However, both cases and controls were equally subject to the possibility of exclusion of a pregnancy from being an index pregnancy (no miscarriages or loss of a pregnancy at <20weeks gestation were used as the index case in either the pre-eclampsia group or the control group). The actual number of the pregnancy classified as "index pregnancy" (e.g. 1^{st}, 2^{nd}, 3^{rd}) was noted.

5.4.4 **Conclusions**

This study identified several key differences between women with a history of pre-eclampsia and women with normotensive pregnancies. Firstly, the differences in blood pressure persist beyond pregnancy and may be the very first marker of evolving cardiovascular disease, therefore in any women with a history of pre-eclampsia, blood pressure should be kept under review. Secondly, this study confirmed what is already known, about the birth characteristics of pregnancies which are associated with a higher degree of vascular dysfunction, specifically a lower birthweight and earlier gestation of delivery. Thirdly, what this study adds to the current literature, is the finding of decreased flow-mediated dilatation at a later age, many years after index pregnancy, even after adjusting for age, BMI and blood pressure. Pre-eclampsia is an independent risk factor for impaired endothelial function in women with a more remote history of the condition. When one considers the natural history of cardiovascular disease and its evolution, one might naturally expect measurement of endothelial function (as assessed by FMD) to show evidence of change before vessel stiffness (as assessed by AIx and cfPWV) or evidence of

atherosclerotic change in the carotid artery (as assessed by cIMT). Given this context, the difference in FMD is perhaps less unexpected, however it has not previously been reported in women this long after pregnancy to the best of the author's knowledge.

This study lends weight to the role for vascular studies in risk stratification for women with a history of pre-eclampsia. Such studies can reveal evidence of subclinical vascular damage and could be invaluable as an adjunct in the overall comprehensive assessment of risk in these women.

6 Biomarkers in women with a history of pre-eclampsia

6.1 Introduction

The link between cardiovascular disease and pre-eclampsia has been well established, however, the underlying mechanisms of this relationship are still not well understood. Confirmation of a history of pre-eclampsia has been recommended in guidelines for cardiovascular disease in women (89), and monitoring of blood pressure in these women has also been suggested as blood pressure is known to be increased in women after pre-eclampsia. However, when we consider that there is a continuum in the evolution of cardiovascular disease (423), there may be crucial time points, even prior to later development of hypertension, where we might intervene in detecting a woman's cardiovascular risk. Vascular dysfunction (as identified in study participants in the previous chapter) is an early marker of cardiovascular risk, correlating with future risk of cardiovascular events and precedes structural vascular damage (424).

It is hoped that biomarkers may emerge to play a key role in the identification of early disease and add to a woman's own personalised risk assessment of likelihood of developing cardiovascular disease. Endothelial dysfunction and inflammation are key components of both pre-eclampsia and cardiovascular disease. Biomarkers relating to these processes have been studied extensively, including their potential role in prediction of pre-eclampsia and assessment of disease severity. Indeed, with regard to endothelial dysfunction there are several important mediators which are upregulated in pre-eclampsia. These include von Willebrand factor, cellular fibronectin, cell adhesion molecules (such as P-selectin, VCAM-1 and ICAM-1) and cytokines (such as TNFα and IL-6) (425). Generalized vasoconstriction as seen in pre-eclampsia may be due in part to nitric oxide deficiency. Oxidative stress from generation of free radicals also contributes to endothelial dysfunction, not only in pre-eclampsia, but also in atherosclerosis (425). Pro-angiogenic effects of VEGF are antagonised by sFlt-1 thus enhancing endothelial dysfunction and contributing to proteinuria and hypertension. PlGF levels in the maternal circulation are also reduced.

Nevertheless, the investigation of biomarkers after hypertensive disorders of pregnancy has not proven to be forthcoming with any clear answers. Biomarkers which are key in the evolution and prediction of pre-eclampsia may not play as important a role in predicting

cardiovascular risk later in life. Variation between studies such as length of time since index pregnancy, varying study populations and sample size further complicate the issue when attempting to generalise and apply the results of these studies to the population as a whole.

There are a few recent systematic reviews and meta-analyses of biomarkers after hypertensive disorders of pregnancy (179;250;426). In pooled analyses, Grand'Maison et al (179) found mean levels of sFlt-1 were slightly higher in women with a history of hypertensive disorder of pregnancy, but VEGF revealed no significant differences between groups. Visser et al also reported on studies investigating biomarkers of angiogenesis. They described a few individual studies with two studies reporting higher VEGF and sFlt-1 levels (251;252) in women with a history of hypertensive disorders of pregnancy. Two studies described higher TNFα levels (256;271), and one study lower TNFα levels (270) in these women.

On examination of biomarkers of inflammation, Grand'Maison et al (179) reported similar levels of ICAM-1 and VCAM-1 between the groups of women. Visser et al (250) also showed no significant difference overall in mean ICAM levels between women with a history of hypertensive pregnancy and controls. Mean VCAM levels similarly showed no significant differences between groups. Overall, IL-6 levels and E-selectin levels were higher in women with a history of hypertensive pregnancy disorders. A study by Freeman et al (256) which is referenced in one of the pooled analyses (250), found an increased IL-6/IL-10 ratio in women 20 years after index pregnancy. Plasma ICAM-1 and VCAM-1 levels were also higher in this group of women in this study.

In addition to the more conventional biomarkers previously studied in relation to cardiovascular disease and hypertensive disorders of pregnancy, such as inflammatory markers and adhesion molecules, this chapter also sought to investigate markers of cardiovascular damage and collagen turnover. These included high sensitivity cardiac troponin (hsTnT) and N-terminal pro brain natriuretic peptide (NT-proBNP) as markers of cardiovascular damage, and carboxy-terminal telopeptide (C1TP), carboxy-terminal propeptide of type 1 procollagen (P1CP) and tissue inhibitor of metalloproteinase-1 (TIMP-1) as markers of collagen turnover.

NT-proBNP and cardiac troponin T (TnT) have both been found to be independent predictors of incident cardiovascular disease and coronary heart disease in a study of

asymptomatic individuals with multi-ethnic background (427). NT-proBNP is established as an important biomarker in heart failure (428). It has also been found to be significantly higher in women with a history of pre-eclampsia in comparison with controls in a systematic review and meta-analysis of shared biomarkers between diastolic heart failure and pre-eclampsia (429). High sensitivity cardiac troponin (hsTnT) has been investigated in heart failure (430;431) and has also been found to be independently associated with incident coronary heart disease, death and heart failure in a large cohort (432).

Markers of collagen turnover have also been linked to several different areas of cardiovascular disease (433). Cardiac fibroblasts (CFBs) produce extracellular matrix proteins to maintain the integrity of the cardiac extracellular matrix (ECM). They also produce regulatory proteins such as matrix metalloproteinases (MMPs) and their inhibitors (TIMPs). TIMP-1 levels rise in heart disease; it is usually expressed at low levels in the healthy heart (433). In a study of TIMP-1, C1TP and P1CP in hypertensive patients, all three were significantly increased (434). More specific to pre-eclampsia, TIMP-1 levels have previously been found to be increased (435;436), in these women.

Urinary proteomic biomarkers have been developed for early diagnosis in several diseases such as stroke (437), chronic kidney disease (438), coronary artery disease (439), and pre-eclampsia (340). Coronary artery disease (CAD_{238}) and kidney disease (CKD_{273}) panels were analysed as described in this chapter to investigate whether there were any differences between cases and controls. In addition, the pre-eclampsia panel was included to establish if it was able to differentiate between cases and controls at a much later time point after pregnancy.

The overall aims of this chapter were to investigate whether there were any significant differences in biomarkers such as inflammatory cytokines, adhesion molecules, markers of cardiac damage, markers of collagen turnover and urinary proteomic panels in women with pre-eclampsia and normotensive controls years after delivery.

6.2 Methods

6.2.1 Patient recruitment

For the biomarker studies discussed in this chapter, samples were studied from two separate cohorts: women who had participated in GS:SFHS and women who attended for the COPS vascular studies. Generation Scotland study samples were prepared at the time of the study visit as mentioned in section 6.2.3.1.

For women who attended for the COPS vascular study, their samples were prepared at the time of the study visit as outlined in Chapter 2.

6.2.2 Identification of samples for biomarker studies

6.2.2.1 *The Generation Scotland cohort*

Women with a history of pre-eclampsia had already been identified as part of the Generation Scotland record linkage study in Chapter 3. Of the pre-eclampsia cases identified, 329 had samples available and they were each matched to two normotensive controls (658 controls in total). Each case therefore had a corresponding control 1 and control 2. Matching was based on smoking history, year of birth, BMI and systolic blood pressure. From this, a matched set of 56 cases with their 112 corresponding controls for biomarker studies was derived. Cases were chosen according to median time since index (pre-eclamptic) pregnancy. Cases were taken from the right and left of the median and for the final cohort, by the time of biomarker studies, median length of time since index pregnancy was 32yrs. Of the total 168 participants (56 cases and 112 controls) identified, 4 serum samples were not available (1 case, 2 x control 1, and 1 x control 2). Due to the methods of statistical analysis for a matched pairs design, biomarker data was excluded from analysis for the missing case and its corresponding controls. The Generation Scotland cohort underwent biomarker analysis on the Randox Investigator platform (section 6.2.4.1) and ELISA studies (section 6.2.4.2).

6.2.2.2 *COPS vascular study cohort*

Of the COPS vascular study participants, 40 cases and 40 controls were picked for biomarker studies. These samples were not specifically matched as the COPS vascular study was still recruiting at the time biomarker studies began. Serum samples were available for analysis on the Luminex® MAGPIX® platform on 37 cases and 37 controls and urine samples were available for urinary proteomic analysis in 38 cases and 38 controls.

6.2.3 **Sample preparation**

6.2.3.1 *Generation Scotland sample preparation*

Generation Scotland samples were collected and stored according to standard operating procedures. A laboratory information management system (LIMS) was utilised and samples were barcoded. The barcode acted as a unique identifier and linked all data collected with blood and urine samples obtained at the study visit. A total of 35ml of blood was collected from GS:SFHS participants: 1 x 9ml EDTA (lavender top), 3 x 5ml serum gel separator (SST) (gold top), 1 x 9ml ACD (yellow top), 1 x 2ml fluoride oxalate (grey top). The fluoride oxalate and 1 SST gold top tube were forwarded to local NHS biochemistry laboratories for biochemistry analysis (glucose, urea and electrolytes, lipids). Samples were then forwarded to the local Generation Scotland site, which for Glasgow was BHF GCRC. 1 x 9ml EDTA was stored at -80^{o}C until sent to the Wellcome Trust Clinical Research Facility in Edinburgh for further processing. Cryotubes were used for urine 4 x 1ml aliquots and serum (prepared from gold top SST tubes) 4 x 1ml aliquots, all stored at -80^{o}C.

For the purposes of the studies outlined in this chapter, requested serum samples were thawed before processing and Mr Jim McCulloch at BHF GCRC oversaw the forwarding of a 150ul aliquot was to Dr Anne Marie Jennings at Randox for processing as described in section 6.2.4.1, and a 300ul aliquot to Dr Susanna Ravassa at the University of Navarra, Pamplona for ELISA studies mentioned in section 6.2.4.2.

6.2.3.2 *COPS study sample preparation*

Samples were collected from the antecubital fossa, centrifuged at 2500 rpm for 15 minutes at 4^{o}C, and for the purposes of future biomarker analysis 0.5µl aliquots of serum and plasma were placed in cryotubes and stored at -80^{o}C. Otherwise, a lavender EDTA tube for future DNA analysis and a blue tempus RNA tube were also stored at -80^{o}C. Urine was stored in 3 x 1ml cryotube aliquots. Samples were then thawed prior to testing. A 50ul aliquot of serum was forwarded to Ms Liliya Sharafetdinova at the BHF GCRC for processing on the Luminex® MAGPIX® platform, as described in section 6.2.4.3. A 700ul aliquot of urine was forwarded to the University of Glasgow ICAMS Proteomics Laboratory for urinary proteomic analysis, also performed by Ms Liliya Sharafetdinova as outlined in section 6.2.4.4 and overseen by Dr William Mullen.

6.2.4 **Biomarker studies**

6.2.4.1 *Randox Investigator platform studies*

The Randox Evidence Investigator platform was used to assess biomarkers in the Adhesion Panel (VCAM-1, ICAM-1, E-selectin, P-selectin and L-selectin) and in the Cytokine High Sensitivity Array Panel (IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, VEGF, IFNγ, EGF, MCP-1 and TNFα) . Samples were analysed by Dr Ann Marie Jennings (Randox Laboratories, County Antrim, UK), using Biochip Array Technology.

A 9x9mm biochip was used to analyse multiple different biomarkers at once for each sample. This was achieved through the pre-fabrication of discrete test regions (DTRs) with immobilised antibodies which were specific for a different biomarker at each region, therefore one biochip (with multiple markers) was used for each sample. The biochips were arranged in carriers with 3x3 biochips, and these were then handled in trays with a capacity of 6 carriers for each tray. Chemiluminescent immunoassay technology was used, with an increase in chemiluminescence signal occurring if there was an increased level of the biomarker present. The mechanism for this was that increased levels of the marker would enhance the amount of binding of antibody labelled with horseradish peroxidase (HRP), which would in turn enhance the amount of signal emitted. Digital imaging technology picked up the light signals from each region of the biochip and compared them against calibrated values. The concentration of the biomarker was calculated from this information. Biochips specific for the Adhesion molecules panel and the Cytokine High Sensitivity Array Panel were used for the purposes of this study.

Table 6.1 Sensitivities and assay precision for Randox Cytokine High Sensitivity Array and Adhesion Molecules array

Analyte	Sensitivity	Reported intra-assay precision	Reported inter-assay precision
Cytokine high sensitivity array			
IL-1α	0.19 pg/ml	9.7-11.4%	8.9-15.5%
IL-1β	0.26 pg/ml	7.4-9.3%	8.7-11.8%
IL-2	0.90 pg/ml	5.8-7.8%	6.5-8.2%
IL-4	2.12 pg/ml	8.1-9.5%	8.6-11.8%
IL-6	0.12 pg/ml	7.8-11.9%	7.4-8.4%
IL-8	0.36 pg/ml	7.0-9.4%	9.2-11.1%
IL-10	0.37 pg/ml	5.6-6.8%	6.5-7.5%
VEGF	1.53 pg/ml	7.3-10.8%	7.2-12.0%
IFNγ	0.44 pg/ml	7.4-10.1%	6.4-11.4%
EGF	1.04 pg/ml	9.2-9.6%	8.5-11.7%
MCP-1	0.66 pg/ml	5.8-12.2%	7.2-12.8%
TNFα	0.59 pg/ml	7.1-12.7%	6.7-8.6%
Adhesion molecules array			
VCAM-1	4.1 ng/ml	7.7-9.2%	5.9-9.6%
ICAM-1	1.7 ng/ml	6.4-9.4%	3.5-8.3%
E-Selectin	0.1 ng/ml	5.2-7.4%	7.5-8.6%
P-Selectin	1.9 ng/ml	6.1-9.6%	4.8-7.2%
L-Selectin	3.2 ng/ml	6.1-9.1%	7.7-13.4%

6.2.4.2 *ELISA studies*

Markers of collagen turnover and cardiac damage were analysed by Dr Susana Ravassa at the University of Navarra, Pamplona, Spain, and tests were carried out on available kits according to manufacturer protocols and specifications. Carboxy-terminal telopeptide of type 1 collagen (C1TP) was tested by enzyme immunoassay (Orion Diagnostica, Espoo, Finland), carboxy-terminal propeptide of type 1 procollagen (P1CP) was analysed by ELISA (Quiadel Corporation, San Diego, USA) and tissue inhibitor of metalloproteinase 1 (TIMP-1) was analysed by ELISA (GE Healthcare, Little Chalfont, UK).

For biomarkers of cardiac damage, high sensitivity cardiac troponin T (TnT) was analysed by ELISA (Roche Diagnostics, Basel, Switzerland) and N-terminal prohormone of brain natriuretic peptide (NT-proBNP) was similarly tested by ELISA (Roche Diagnostics, Basel, Switzerland).

6.2.4.3 *Luminex® MAGPIX® platform studies*

Samples were analysed using the Merck MILLIPLEX® MAP Human Th17 Magnetic Bead Panel and results analysed using the Luminex® MAGPIX® system which uses light-emitting diode /charge-coupled device image-based detection.

Reagents were prepared according to the manufacturer instructions and specifications. All reagents were warmed to room temperature before use. Assay buffer (200µl) was added to each well of the experiment plate. This was sealed and mixed on a plate shaker at room temperature for 10 minutes. The buffer was then removed from the wells thoroughly by inverting the plate onto absorbent towels. Control or standard (25µl) was added to appropriate wells and 25µl of assay buffer was then added to background and sample wells. Appropriate matrix solution (25µl) was added to control, standards and background wells. Samples (25µl) were added to the sample wells and beads (25µl) were then added to each well. The plate was sealed, covered with foil and incubated for 16-18 hours overnight at 4^{o}C. Well contents were removed and 200µl wash buffer was used to wash the plate twice. Detection antibodies (25µl) were added to each well and the plate was sealed, covered in foil and incubated at room temperature for an hour. Streptavidin-Phycoerythrin (25µl) was added to each well. The plate was then sealed, covered with foil and incubate at room temperature for 30 minutes. Well contents were removed and 200µl of wash buffer was again used to wash the plate twice. Drive fluid (150µl) was added to all wells and beads were re-suspended on a plate shaker for 5 minutes. The plate was then run on the MAGPIX® system. Analyte sample concentrations were calculated using Median Fluorescent Intensity (MFI) data.

Table 6.2 Assay information for the Merck MILLIPLEX® MAP Human TH17 Panel

Analyte	Minimum Detectable Concentration (MinDC)	Reported intra-assay precision	Reported inter-assay precision
IL-17F	0.009 ng/ml	2%	10%
GM-CSF	0.146 ng/ml	5%	13%
IFNγ	1.8 pg/ml	4%	13%
IL-10	0.3 pg/ml	3%	11%
CCL20/MIP3α	2.2 pg/ml	5%	10%
IL-12p70	1.1 pg/ml	3%	8%
IL-13	2.4 pg/ml	3%	9%
IL-15	2.7 pg/ml	4%	12%
IL-17A	2.1 pg/ml	3%	13%
IL-22	0.021 ng/ml	4%	8%
IL-9	6 pg/ml	3%	5%
IL-1β	2.1 pg/ml	4%	11%
IL-33	6.3 pg/ml	5%	11%
IL-2	5.1 pg/ml	3%	11%
IL-21	2 pg/ml	3%	11%
IL-4	0.009 ng/ml	4%	11%
IL-23	0.098 ng/ml	3%	9%
IL-5	1.2 pg/ml	4%	7%
IL-6	1.7 pg/ml	5%	7%
IL-17E/IL-25	0.099 ng/ml	5%	10%
IL-27	0.063 ng/ml	4%	10%
IL-31	0.021 ng/ml	4%	8%
TNFα	0.9 pg/ml	4%	11%
TNFβ	0.021 ng/ml	3%	7%
IL-28A	0.038 ng/ml	3%	13%

6.2.4.4 *Urinary proteomic studies*

Prior to analysis samples were thawed and a 700µl aliquot of urine was then diluted with a 700µl volume of 2 mol/L urea and 10 mmol/L NH_4OH containing 0.02% sodium dodecyl sulphate (SDS). Samples were spun at a temperature of 4^{o}C for 1 hour at 3000g using a Centrisart ultracentrifugation device (Sartorius, Göttingen, Germany). This removed proteins with a molecular weight of greater than 20 kDa. Salts, urea and electrolytes were then removed by running the filtrate through a PD-10 desalting column (Amersham Bioscience, Buckinghamshire, UK) and peptide elution was performed with 0.01% aqueous NH_4OH. Samples were then lyophilised, suspended in high-performance liquid chromatography-grade water, and stored at 4^{o}C prior to analysis by CE-MS.

CE-MS was performed by coupling a P/ACE MDQ capillary electrophoresis system (Beckman Coulter, Fullerton, USA) to a micro time-of-flight (micro-TOF) mass spectrometer (Bruker Daltonic, Bremen, Germany). First the silica capillary was rinsed for 3 minutes with a buffer of 30% methanol and 0.5% formic acid in HPLC grade water. Approximately 700nl sample was injected into the capillary. A charge of +30kV was applied at the capillary inlet in order to separate peptides, and the entire length of the 90cm capillary was kept at a constant temperature of 35^{o}C. Before a further sample can be processed, the capillary must first be rinsed with 0.1M NaOH, then water for 5 minutes followed by running buffer.

Peptides were then identified by their mass/charge ratio. The electro-ionisation (ESI) sprayer (Agilent Technologies, California, USA) was grounded and the potential was fixed at -4.5kV. Every 3 seconds, spectra were accumulated over a range of mass-to-charge ratios (350-3000). The mean was calculated afterwards. Due to the vast amount of information which is produced with each CE-MS run, several further steps were necessary in data processing. Peaks were detected and their charges calculated by specialised software (Mosaiques Visu) (440). The data were then deconvoluted which resulted in a single mass being recorded by the software following analysis of many spectral peaks which were created from the same molecule at different charge states.

Due to potential confounding (both from patient and sample analytical issues), it was important to normalise the data for CE migration time and signal intensity. There are 29 “housekeeping” peptides which function as internal standards and are unaffected by disease states, age or gender (441). These were used to normalise the data. The end result of processing was a peak list which characterised each peptide based on its CE migration time, molecular mass and signal intensity.

For all samples which passed quality control, the peptide information generated was entered into a Microsoft SQL database to facilitate comparison with other samples. Features between samples were considered the same if mass deviation was < ±50ppm at 800 Da and increased to ±75ppm for 20kDa peptides. A unique identification number was assigned to each peptide.

In order to perform statistical analysis of CE-MS results between patient groups, a single numerical value, the “classification factor” was derived using MosaCluster software. This uses support vector machine (SVM) based mathematical modelling. Several urinary

proteomic panels have been derived for use in research and for the purposes of the studies in this chapter, the CKD_{273} (438), CAD_{238} (439) and pre-eclampsia (340) panels were used.

6.2.5 **Statistical analysis**

Statistical analyses were performed using Minitab v17 (Minitab Inc, State College, PA, USA) and SPSS v22 (IBM Corp, Armonk, New York, USA). Normality of the distribution was assessed using the Kolmogorov-Smirnov test and visual inspection of histograms and plots.

No outliers were removed for the COPS vascular study cohort. For the Generation Scotland cohort, a minimal number of outliers were removed for reasons of suspicion of technical artefact in the following samples: NT-proBNP (1 case), hsTnT (1 case), IFNγ (1 case), IL-10 (2 controls), IL-2 (1 case), IL-6 (1 case, 1 control), IL-8 (1 control), TNFα (1 control). For any biomarker samples with results which registered as less than the limit of detection (LOD), a value of half of the limit of detection was used (LOD/2).

Data were transformed as required and expressed as mean ± standard deviation if normally distributed and as median (inter-quartile range) if distribution did not normalise following transformation. For matched pairs comparison in the Generation Scotland cohort, for continuous data, the mean of the two control values was used to represent controls overall, thus, for each case there was one corresponding control value. Paired t-test was used to compare normally distributed data. If data were not normally distributed and failed to normalise on transformation, related samples Wilcoxon signed rank test was used. Fisher's exact test was used to compare categorical data. For independent samples comparison for the unmatched COPS vascular study cohort and the second stage of the Generation Scotland cohort tests, independent samples t-test was carried out on normally distributed data, and if data were not normally distributed and did not normalise on transformation, comparisons were made using the Mann-Whitney U test. Comparison of categorical data was performed using Chi-squared test, or Fisher's exact test as appropriate. Correlations were analysed using Pearson's or Spearman's correlation coefficient as required. Further analysis was performed using multivariate linear regression where appropriate. This was considered to be an exploratory study, therefore a p-value of <0.05 was used as the cut-off for significance.

6.3 **Results**

Table 6.3 Generation Scotland study patient characteristics for all original pre-eclampsia cases and matched controls

Parameter	Control (n=658)	Case (n=329)	P-value
Age (yrs)	53 (11)	53 (10)	0.125
BMI (kg/m^2)	27 (6.8)	27 (8)	0.126
SBP (mmHg)	129 (21)	130 (23)	< **0.001**
DBP (mmHg)	81 (12)	82 (12)	< **0.001**
Glucose (mmol/L)	4.6 (0.5)	4.6 (0.7)	0.891
HDL cholesterol (mmol/L)	1.54 ± 0.3	1.57 ± 0.4	0.579
Total cholesterol (mmol/L)	5.38 ± 0.8	5.27 ± 1.1	0.072
Sodium (mmol/L)	140 (2)	140 (2)	0.324
Potassium (mmol/L)	4.1 (0.3)	4.1 (0.4)	0.996
Urea (mmol/L)	4.8 ± 0.9	4.9 ± 1.3	0.852
Creatinine (μmol/L)	67 (10)	66 (13)	0.673
Birth weight index pregnancy (grams)	3454.5 (472.5)	3300.0 (868.0)	<**0.001**
Gestation at delivery index pregnancy (weeks)	40 (1)	39 (2)	<**0.001**
Current hypertension	61/606 (10.1%)	94/322 (29.2%)	**0.000**
Current diabetes	8/606 (1.3%)	12/322 (3.7%)	**0.029**
Current smoker	93/609 (15.3%)	46/320 (14.4%)	0.772

Results are presented as mean ± standard deviation or median (inter-quartile range) as appropriate. Data were analysed using paired t-test or Wilcoxon signed rank. Categorical data were analysed using Fisher's exact test.
Subjects were matched for Smoking status, year of birth, BMI and systolic blood pressure.
BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, HDL = high density lipoprotein

In the overall cohort of 329 cases and 658 controls, women with a history of pre-eclampsia had a statistically significantly higher systolic blood pressure and diastolic blood pressure later in life and they were more likely to have given birth to lower birth weight babies and at an earlier gestation (see Table 6.3). Descriptive statistics for the cohort which was chosen from this group for biomarker studies can be seen in Table 6.4.

Table 6.4 Characteristics of Generation Scotland biomarker studies cohort

Parameter	Controls (n=110)	Cases (n=55)	P-value
Age (yrs)	56 (2.5)	56 (3.0)	0.819
BMI (kg/m^2)	26 (7.5)	26 (8)	0.060
SBP (mmHg)	134 ± 16	136 ± 18	< **0.001**
DBP (mmHg)	82 ±9	84 ± 10	0.216
Glucose (mmol/L)	4.65 ± 0.38	4.86 ± 0.86	0.108
HDL cholesterol (mmol/L)	1.6 ± 0.3	1.5 ± 0.4	0.179
Total cholesterol (mmol/L)	5.5 ± 0.7	5.7 ± 1.1	0.276
Sodium (mmol/L)	140.4 ± 1.4	139.8 ± 1.8	0.076
Potassium (mmol/L)	4.1 ± 0.3	4.0 ± 0.4	0.466
Urea (mmol/L)	5.0 ± 0.8	5.1 ± 0.9	0.795
Creatinine (μmol/L)	67.4 ± 7.0	66.4 ± 10.7	0.590
Birth weight index pregnancy (grams)	3425 (560)	3390 (850)	0.221
Gestation at delivery index pregnancy (weeks)	40 (1)	40 (1)	0.058
Current hypertension	14/98 (14.3%)	15/53 (28.3%)	0.051
Current diabetes	2/98 (2.0%)	3/53 (5.7%)	0.345
Current smoker	11/99 (11.1%)	5/53 (9.4%)	1.000

Results are presented as mean ± standard deviation or median (inter-quartile range) as appropriate.
Results are from paired t-test or Wilcoxon signed rank. The mean of controls was used to provide one overall value for controls for this test. Subjects were matched for Smoking status, year of birth, BMI and systolic blood pressure. Categorical data were analysed using Fisher's exact test.
BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, HDL = high density lipoprotein

The difference in systolic blood pressure between cases and controls were also statistically significantly different in this smaller cohort whereas birth characteristics did not remain statistically significantly different. Current diagnosis of hypertension and diabetes were more prevalent in the overall cohort of 329 cases and 658 controls, however these were not significantly different in the smaller biomarker studies cohort.

Cases and controls had been matched for smoking so there was no significant difference between cases and controls for this parameter (see Tables 6.3 and 6.4).

For the COPS vascular studies cohort, pre-eclampsia cases had significantly higher systolic blood pressure and diastolic blood pressure than controls (see Table 6.5). This was

consistent across sitting blood pressure, supine blood pressure and the calculated central blood pressures on pulse wave analysis. Mean heart-rate adjusted augmentation index was also significantly higher in cases, along with mean pulse wave velocity. Gestation at delivery and birth weight of index pregnancy were both statistically significantly lower in cases and women with pre-eclampsia were more likely to have a diagnosis of hypertension or have a blood pressure measurement in the hypertensive range at time of the COPS vascular study visit.

Table 6.5 Characteristics of the COPS vascular study cohort

Parameter	Controls (n=40)	Cases (n=40)	P-value
Age (yrs)	46 (9)	46 (10)	0.761
BMI (kg/m^2)	27.3 ± 4.6	27.5 ± 4.4	0.876
Height (cm)	163.2 ± 7.0	162.2 ± 6.7	0.524
Weight (kg)	72.7 ± 12.8	72.2 ± 11.7	0.855
Mean* SBP (mmHg)	122.7 ± 9.4	130.2 ± 15.5	**0.012**
Mean* DBP (mmHg)	77.5 ± 7.6	82.5 ± 8.4	**0.006**
Resting Heart Rate (bpm)	70 ± 9	72 ± 11[#]	0.477
Total cholesterol (mmol/L)	5.22 ± 0.82	5.27 ± 0.92	0.768
Triglycerides (mmol/L)	1.03 (0.95)	1.0 (0.70)[†]	0.472
HDL cholesterol (mmol/L)	1.3 (0.55)[‡]	1.5 (0.47)[§]	0.499
Supine SBP (mmHg)	117 ± 10	125 ± 15	**0.005**
Supine DBP (mmHg)	72 ± 7	77 ± 9	**0.010**
Mean Central SBP (mmHg)	108 ± 10	116 ± 14	**0.004**
Mean Central DBP (mmHg)	73 ± 7	78 ± 9	**0.006**
Mean AIx @75	21.4 ± 8.5	25.5 ± 9.0	**0.041**
Mean PWV (m/s)	7.08 ± 0.99	7.75 ± 1.71	**0.036**
Mean cIMT CCA	0.630 ± 0.114	0.640 ± 0.127	0.713
Mean cIMT Bulb	0.696 ± 0.139	0.698 ± 0.141	0.956
Mean cIMT ICA	0.573 ± 0.154[†]	0.561 ± 0.141[#]	0.720
FMD %	6.41 ± 3.18[a]	5.95 ± 3.05[b]	0.541
Age at index pregnancy (yrs)	30 ± 6	29 ± 4	0.229
Time since index pregnancy (yrs)	16.5 (17)	19.0 (16)	0.330
C-section	13 (32.5%)	14 (35%)	0.813
Gestation at delivery (wks)	40 (2.0)	38 (3.7)	**0.000**
Birth weight (g)	3446.7 ± 511	2898.8 ± 756	**0.000**
Current hypertension diagnosis	3 (7.5%)	11 (27.5%)	**0.037**
Hypertensive range blood pressure at COPS study visit	1 (2.5%)	8 (20%)	**0.029**
Current diabetes	0 (0%)	1 (2.5%)	1.000
Current smoking	6 (15%)	3 (7.5%)	0.481

Values are expressed as mean ± standard deviation or median (inter-quartile range) depending on distribution of the data. Two-sample t-test or Mann-Whitney U tests were performed accordingly. Chi-test/Fisher's exact test was performed for categorical variables.

***Mean of 2nd and 3rd blood pressure readings.**

 # For this group n=38, † this group n=39, ‡ this group n= 29, §this group n= 36

[a] this group n=33, [b]this group n=37.

BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, HDL = high density lipoprotein

6.3.1 Generation Scotland cohort

6.3.1.1 *Results of biomarker studies*

Analysis of biomarker studies in the Generation Scotland cohort was conducted as paired tests according to original matching criteria, matching each case to the mean of its two controls. In addition I also analysed the dataset as two independent samples so that I was able to adjust for a wider range of co-variates.

Table 6.6 Results from Generation Scotland biomarker study group when analysed as matched cases and controls

	Controls (n=55)*	Cases (n=55)	P-value
Cytokine high sensitivity array			
IL-1α (pg/ml)	0.225 (0.245)	0.195 (0.145)	**0.015**
IL-1β (pg/ml)	0.98 (0.69)	0.98 (1.09)	0.090
IL-2 (pg/ml)	1.28 (1.55)	0.45 (2.0)	0.736
IL-4 (pg/ml)	1.94 ± 0.56	1.74 ± 0.55	**0.017**
IL-6 (pg/ml)	2.18 ± 1.26	1.91 ± 1.64	**0.030**
IL-8 (pg/ml)	17.37 ± 14.5	17.68 ± 16.3	0.594
IL-10 (pg/ml)	0.764 ± 0.322	0.816 ± 0.540	0.749
IL-6/IL-10 ratio	3.10 ± 1.93	2.89 ± 2.69	0.075
VEGF (pg/ml)	128.14 ± 79.73	197.72 ± 179.19	0.085
IFNγ (pg/ml)	0.31 (0.26)	0.22 (0.03)	**0.000**
EGF (pg/ml)	77.81 ± 36.88	81.87 ± 56.30	0.618
MCP-1 (pg/ml)	234.9 ± 66.2	220.9 ± 84.9	0.338
TNFα (pg/ml)	3.01 ± 0.84	2.90 ± 1.00	0.494
Adhesion molecules array			
VCAM-1 (ng/ml)	513.9 ± 106.0	469.9 ± 125.6	**0.039**
ICAM-1 (ng/ml)	278.53 ± 73.51	250.35 ± 74.98	**0.043**
E-Selectin (ng/ml)	16.47 ± 5.55	16.03 ± 5.33	0.653
P-Selectin (ng/ml)	190.2 ± 41.6	192.2 ± 57.8	0.840
L-Selectin (ng/ml)	1548.5 ± 289.6	1487.4 ± 408.7	0.378
ELISA studies			
NT-proBNP (ng/L)	67.93 ± 33.65	68.00 ± 35.69	0.946
hsTnT (μg/L)	0.00582 ± 0.0023	0.00579 ± 0.0022	0.939
P1CP (ng/ml)	82.55 ± 26.28	84.06 ± 28.76	0.797
C1TP (ng/ml)	3.07 ± 0.87	2.81 ± 0.96	0.169
TIMP-1 (ng/ml)	388.46 ± 67.13	415.92 ± 91.21	0.094

*** Mean of control 1 and control 2 used = 55 data entries for analysis. Results are presented as mean ± standard deviation or median (inter-quartile range) as appropriate.**

On paired analysis, IL-1α, IL-4, IL-6 and IFNγ were significantly lower in cases than controls. VCAM-1 and ICAM-1 were also statistically significantly lower in cases. The IL-6/IL-10 ratio was lower in cases than controls, but this was not statistically significant.

Table 6.7 Results from Generation Scotland biomarker study group when analysed as two independent samples

	Controls (n=107)	Cases (n=55)	P-value
Cytokine high sensitivity array			
IL-1α (pg/ml)	0.20 (0.13)	0.20 (0.15)	0.852
IL-1β (pg/ml)	1.11 (1.23)	0.98 (1.09)	0.192
IL-2 (pg/ml)	0.45 (1.89)	0.45 (2.00)	0.418
IL-4 (pg/ml)	1.78 (0.56)	1.62 (0.40)	**0.029**
IL-6 (pg/ml)	2.198 ± 1.759	1.908 ± 1.644	0.208
IL-8 (pg/ml)	12.52 (10.3)	12.69 (11.0)	0.905
IL-10 (pg/ml)	0.66 (0.39)	0.66 (0.36)	0.955
IL-6/IL-10 ratio	3.35 ± 2.96	2.89 ± 2.69	0.210
VEGF (pg/ml)	125.17 ± 93.41	197.72 ± 179.19	**0.008**
IFNγ (pg/ml)	0.27 (0.17)	0.22 (0.03)	**0.000**
EGF (pg/ml)	69 (80)	72 (87)	0.595
MCP-1 (pg/ml)	225 (126)	208 (90)	0.299
TNFα (pg/ml)	3.026 ± 1.206	2.897 ± 1.005	0.498
Adhesion molecules array			
VCAM-1 (ng/ml)	512.52 ± 149.74	469.87 ± 125.57	0.072
ICAM-1 (ng/ml)	277.63 ± 95.37	250.34 ± 74.98	0.091
E-Selectin (ng/ml)	16.34 ± 7.02	16.03 ± 5.33	0.963
P-Selectin (ng/ml)	190.1 ± 57.78	192.2 ± 57.8	0.887
L-Selectin (ng/ml)	1480 (506)	1454 (555)	0.286
ELISA studies			
NT-proBNP (ng/L)	67.28 ± 45.15	68.00 ± 35.69	0.529
hsTnT (μg/L)	0.00571 (0.0022)	0.00575 (0.0023)	0.241
P1CP (ng/ml)	82.02 ± 34.73	84.06 ± 28.76	0.497
C1TP (ng/ml)	3.05 ± 1.22	2.81 ± 0.96	0.307
TIMP-1 (ng/ml)	387.62 ± 95.55	415.92 ± 91.21	**0.047**

Results are presented as mean ± standard deviation or median (inter-quartile range) as appropriate.

On analysis as independent samples, IL-4 and IFNγ were still statistically significantly lower in cases. VEGF was significantly higher in cases and TIMP-1 was also significantly higher in cases. Differences between IL-1α, IL-6 and adhesion molecules VCAM-1 and ICAM-1 were no longer of statistical significance.

6.3.1.2 *Univariate and multivariate analyses*

Interferon-γ did not have any significant correlations with any of the variables investigated.

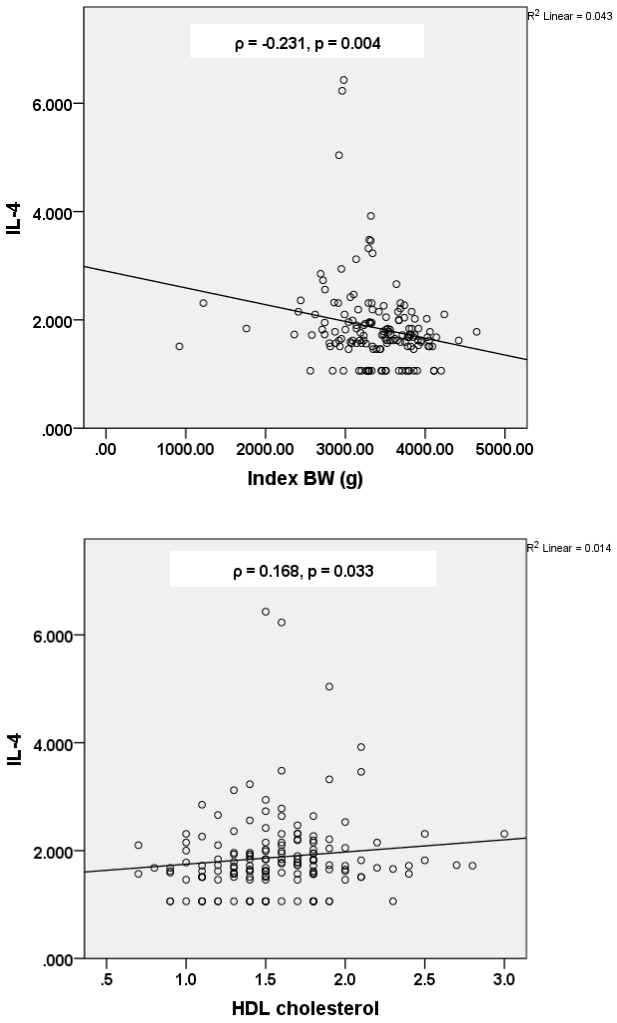

Figure 6.1 Scatterplots of IL-4 against birth weight at index pregnancy and HDL cholesterol. Correlation coefficients were calculated as Spearman's (rho). Regression lines have only been added to indicate direction of relationship.

IL-4 was statistically significantly negatively correlated with birth weight at index pregnancy and positively with HDL cholesterol. Spearman's correlation revealed ρ= -0.231, p=0.004 for birth weight and ρ=0.168, p=0.033 for HDL cholesterol. Multiple regression analysis could not be reported as some of the important assumptions of the test were violated.

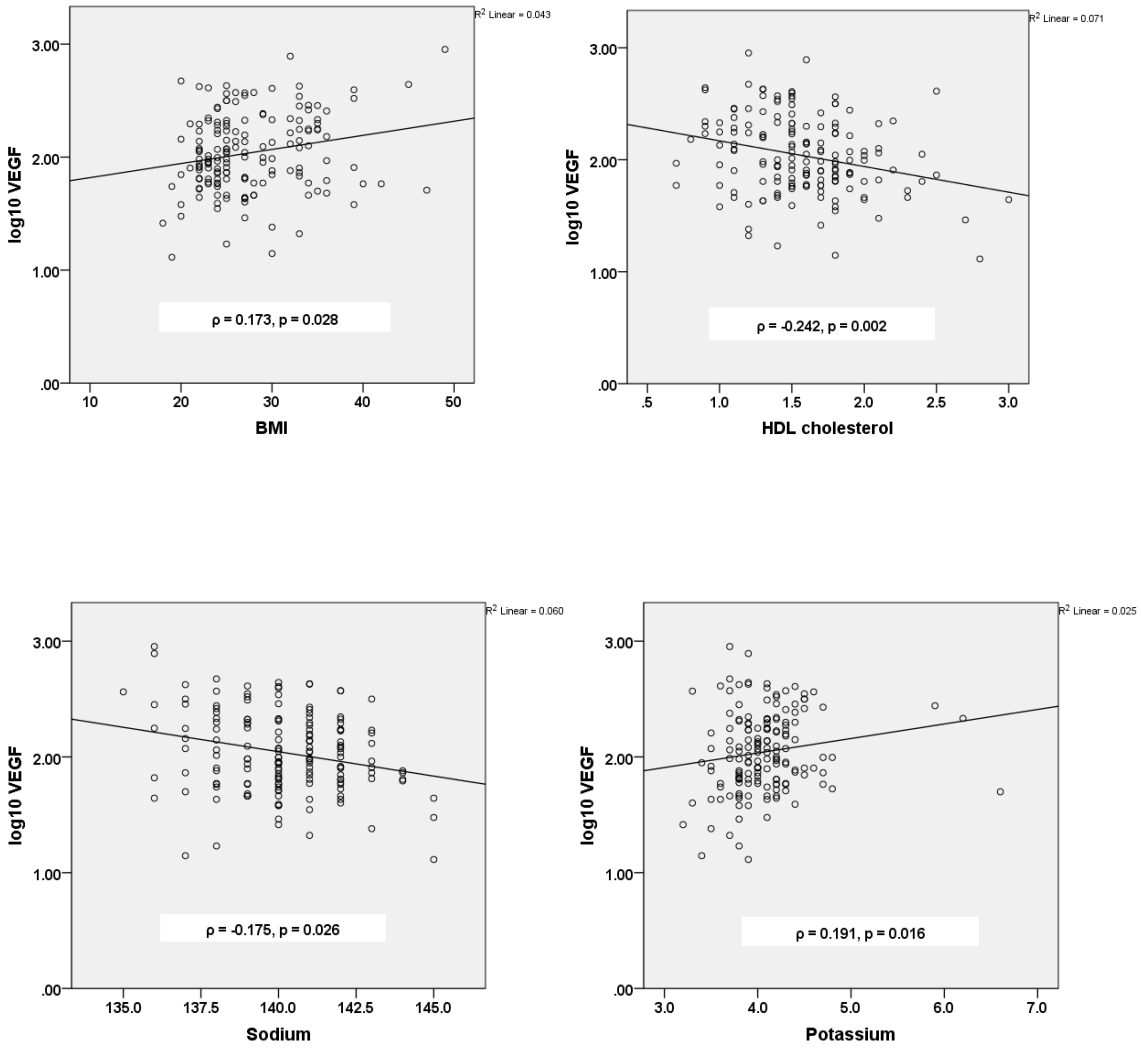

Figure 6.2 Scatterplots of log_{10}VEGF against BMI, HDL cholesterol, sodium and potassium. Regression lines have been added to show direction of relationship.

Log-transformed VEGF was found to be statistically significantly correlated with four variables; BMI, HDL cholesterol, sodium and potassium. VEGF was positively correlated with BMI (ρ=0.173, p=0.028) and potassium (ρ=0.191, p=0.016), and negatively correlated with HDL cholesterol (ρ= -0.242, p=0.002) and sodium (ρ= -0.175, p=0.026). See Figure 6.2.

Multiple regression analysis was then performed, to study the differences in VEGF whilst controlling for potential confounders. In the first model, parameters that were significantly different between cases and controls were entered as co-variates (Table 6.8). In a second model, adjustment for traditional cardiovascular risk factors was made (Table 6.9).

Table 6.8 Model 1 multiple regression for log_{10}VEGF results

Parameter	B	SE_B	β	P-value
Pre-eclampsia	0.123	0.055	0.169	0.025
BMI	0.004	0.005	0.059	0.490
HDL cholesterol	- 0.167	0.073	- 0.192	0.024
Sodium	- 0.031	0.013	- 0.177	0.021
Potassium	0.112	0.060	0.140	0.063

B = unstandardized regression coefficient, SE_B = standard error of the coefficient, β = standardised coefficient.

Table 6.9 Model 2 multiple regression for log_{10}VEGF results

Parameter	B	SE_B	β	P-value
Pre-eclampsia	0.155	0.060	0.212	0.010
Age	0.016	0.016	0.083	0.318
BMI	0.013	0.005	0.217	0.016
SBP	0.000	0.002	0.008	0.930
Total cholesterol	0.016	0.029	0.045	0.589
Diabetes	- 0.048	0.163	- 0.025	0.768
Current smoker	0.095	0.098	0.080	0.336

B = unstandardized regression coefficient, SE_B = standard error of the coefficient, β = standardised coefficient.

Log-transformed TIMP-1 was statistically significantly correlated with HDL cholesterol and creatinine. Due to distribution of HDL and creatinine, nonparametric testing was required. HDL cholesterol was negatively correlated with log_{10}TIMP-1, ($\rho = -0.207$, $p=0.008$), and creatinine was positively correlated ($\rho = 0.185$, $p = 0.018$). See Figure 6.3. Comparison of mean log-transformed TIMP-1, when grouped by hypertension or current smoking was not significant.

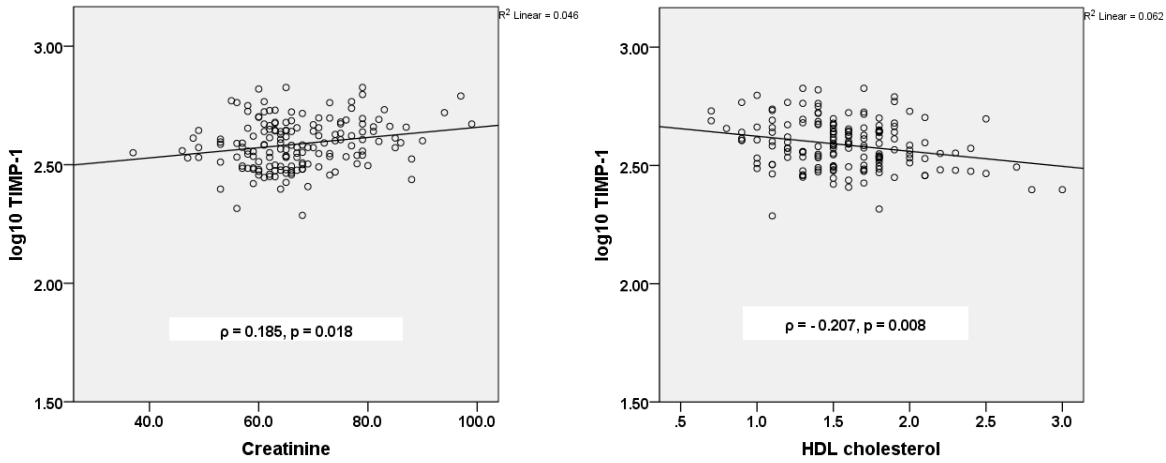

Figure 6.3 Scatterplots of log_{10} TIMP-1 against creatinine and HDL cholesterol

Multiple regression analysis was again performed. Model 1 consisted of pre-eclampsia status, HDL cholesterol and creatinine which had all been significantly different between cases and controls (Table 6.10). Statistically significant independent variables in the model were HDL cholesterol and creatinine. Pre-eclampsia was not a statistically significant determinant. The second model, as before, was made up of pre-eclampsia and classical cardiovascular risk factors. Not one of the independent variables was individually statistically significant (see Table 6.11).

Table 6.10 Model 1 multiple regression for log_{10}TIMP-1 results

Parameter	B	SE_B	β	P-value
Pre-eclampsia	0.032	0.016	0.147	0.053
HDL cholesterol	- 0.051	0.020	- 0.201	0.010
Creatinine	0.002	0.001	0.181	0.019

B = unstandardized regression coefficient, SE_B = standard error of the coefficient, β = standardised coefficient.

Table 6.11 Model 2 multiple regression for log_{10}TIMP-1 results

Parameter	B	SE_B	β	P-value
Pre-eclampsia	0.030	0.018	0.143	0.091
Age	- 0.003	0.005	- 0.061	0.480
BMI	0.001	0.002	0.057	0.532
SBP	0.000	0.001	- 0.030	0.741
Total cholesterol	- 0.002	0.009	- 0.016	0.852
Diabetes	0.037	0.048	0.066	0.450
Current smoker	0.004	0.029	0.010	0.904

B = unstandardized regression coefficient, SE_B = standard error of the coefficient, β = standardised coefficient.

6.3.2 COPS biomarker studies cohort

6.3.2.1 *Results from Luminex® MILLIPORE® MAP studies*

Table 6.12 Results from MAGPIX® platform analysis in the COPS vascular cohort

Biomarker	Controls (n=37)	Cases (n=37)	P-value
IL-17F (ng/ml)	0.0432 ± 0.0136	0.0495 ± 0.0227	0.309
GM-CSF (ng/ml)	0.2997 ± 0.192	0.3546 ± 0.242	0.295
IFNγ (pg/ml)	68.12 ± 22.96	75.25 ± 21.29	0.170
IL-10 (pg/ml)	17.11 ± 5.42	18.83 ± 6.64	0.227
CCL20/MIP3α (pg/ml)	51.14 ± 13.80	60.40 ± 21.36	**0.037**
IL-12p70 (pg/ml)	44.88 ± 13.15	49.70 ± 17.18	0.212
IL-13 (pg/ml)	114.70 ± 42.19	127.80 ± 46.18	0.207
IL-15 (pg/ml)	39.67 ± 13.49	40.90 ± 13.78	0.699
IL-17α (pg/ml)	38.32 ± 13.23	42.19 ± 13.63	0.219
IL-22 (ng/ml)	1.0527 ± 0.231	1.1612 ± 0.381	0.260
IL-9 (pg/ml)	36.83 ± 13.17	39.06 ± 13.36	0.471
IL-1β (pg/ml)	22.718 ± 10.08	23.819 ± 8.21	0.608
IL-33 (pg/ml)	132.2 ± 33.3	147.5 ± 54.4	0.278
IL-2 (pg/ml)	32.94 ± 14.0	34.46 ± 11.9	0.615
IL-21(pg/ml)	81.08 ± 24.71	91.79 ± 32.59	0.122
IL-4 (ng/ml)	0.1471 ± 0.0529	0.1693 ± 0.0890	0.322
IL-23 (ng/ml)	4.316 ± 1.158	4.714 ± 1.61	0.309
IL-5 (pg/ml)	21.85 ± 8.57	23.42 ± 6.84	0.387
IL-6 (pg/ml)	32.09 ± 11.84	33.49 ± 9.97	0.584
IL-17E/IL-25 (ng/ml)	0.898 (0.429)	0.988 (0.461)	0.417
IL-27 (ng/ml)	1.328 ± 0.328	1.436 ± 0.538	0.476
IL-31 (ng/ml)	0.6232 ± 0.165	0.7030 ± 0.291	0.281
TNFα (pg/ml)	40.76 (9.67)	41.04 (13.54)	0.344
TNFβ (ng/ml)	0.2275 ± 0.1095	0.2369 ± 0.0776	0.673
IL-28A (ng/ml)	2.025 (0.544)	2.172 (0.564)	0.405
IL-6/IL-10 ratio	1.86 ± 0.27	1.82 ± 0.30	0.517

Values are expressed as mean ± standard deviation or median (inter-quartile range) depending on distribution of the data. Two-sample t-test or Mann-Whitney U tests were performed accordingly. Data was transformed to attempt normalisation if not normally distributed initially.

Results from Luminex® MAGPIX® studies revealed CCL20/MIP3α to be significantly higher in pre-eclampsia cases (see Table 6.12). IL-6/IL-10 ratio was lower in the pre-eclampsia group which was similar to the finding using the Randox panel in the Generation Scotland (GS) cohort.

Between GS and COPS cohorts, in biomarkers that were similar between panels (IL-1β, IL-2, IL-4, IL-6, IL-10, TNFα, IFNγ) the trend for results were consistent between panels only for one biomarker, IL-10.

6.3.3 **Results from urinary proteomic studies**

Urinary proteomic studies were carried out on 76 available urine samples (38 cases and 38 controls) from the COPS vascular study cohort (Figure 6.4). There were no statistically significant differences between controls and cases. For the CAD_{238} panel mean classification factor for controls was -0.699 (± standard deviation 0.259) and for cases -0.670 (±0.336), p=0.672. For the CKD_{273} panel controls had a mean value of -0.326 (±0.297) vs cases -0.357 (±0.241), p=0.625 and for the pre-eclampsia panel controls had a mean of -1.213 (±0.441) vs cases -1.187 (±0.347) p=0.774.

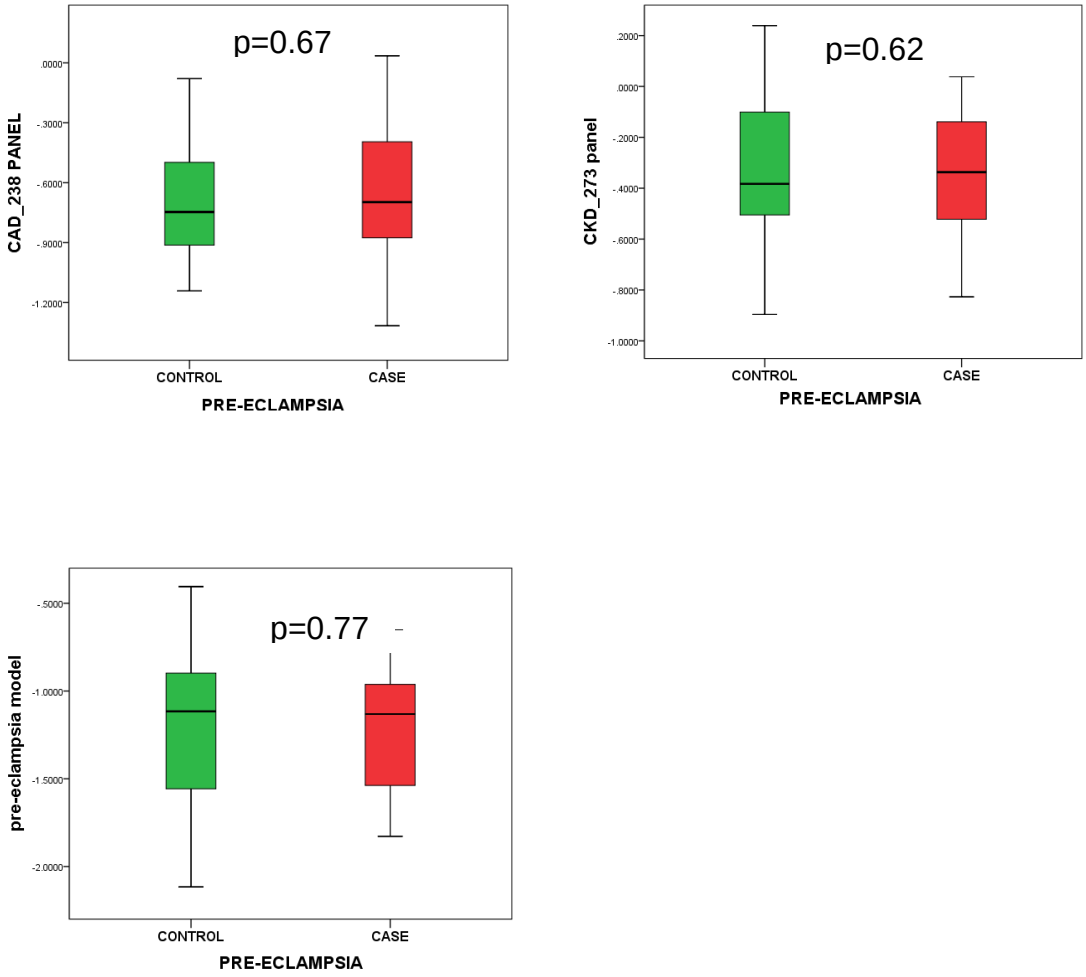

Figure 6.4 Boxplots of urinary proteomic panel results. A) the CAD_{238} panel B) the CKD_{273} panel C) the pre-eclampsia panel. Controls are depicted on the left in green, and cases on the right in red.

6.4 Discussion

6.4.1 Findings

In attempting to explain the vascular findings from chapter 5, most notably the presence of endothelial dysfunction in women with a history of pre-eclampsia, it was hoped that the biomarker studies would yield functional insights. Most interesting of all is probably the unexpected finding of VEGF levels being higher in women with a history of pre-eclampsia. VEGF levels tend to be reduced in women experiencing pre-eclampsia, as sFlt-1 is an inhibitor of VEGF, and sFlt-1 levels are raised in pre-eclampsia and have been found to be raised in women with evidence of endothelial dysfunction after pre-eclampsia (442).

Although previous studies have been contradictory regarding VEGF levels after pre-eclampsia, a recent systematic review and meta-analysis (250) reported a higher median VEGF level in women with a history of hypertensive disorders of pregnancy. However, only two studies relating to VEGF were evaluated and both were small, with the larger describing 29 cases and 32 controls (252). While it is unclear what the specific cause of raised VEGF level is in these women, one must consider the fact that in previous studies of patients with cardiovascular disease, increased levels of circulating VEGF have been reported (244;443).

Different trends in the direction of biomarkers in common between the two cohorts were found. Possible reasons for this include the length of time since index pregnancy and differences in sample preparation. Women from the COPS study were younger and median time since index pregnancy was 16.5 years in controls and 19 years in cases. For the Generation Scotland cohort median time since pregnancy was >30 years. In addition, of note is the fact that samples were from two different cohorts, meaning that they were collected slightly differently, and COPS samples had been collected within months of analysis not years. Another possible reason for inconsistencies between panels is that outliers were removed for one group. There was no indication to remove outliers for the COPS vascular cohort, however, due to the extreme nature of some of the outliers in the GS cohort, and the fact that they could have been due to technical issues, outliers were removed in this group.

The IL-6/IL-10 ratios were not different between cases and controls in either cohort in this study, and they were in fact in the opposite direction to some of the key reports in the field (256).

6.4.2 **Strengths**

For this study 2 controls were matched to each case in the Generation Scotland cohort in an attempt to increase the power. Also, a p-value of <0.05 was used as the cut-off for significance, as the individual biomarker tests were treated as independent hypotheses and this was viewed as a hypothesis generating experiment. In order to confirm the results, additional cohorts would be required.

In addition, this study consisted of two different patient populations from two different time points since pre-eclamptic pregnancy and as such was able to compare biomarkers in this context. In particular, there are few studies which have analysed samples in women so long after pre-eclamptic pregnancy. It is important to consider that where women are positioned on their own cardiovascular disease continuum will vary as they get older, and by simultaneously evaluating women who are all at differing time-points since index pregnancy, one might be able to observe alterations in biomarkers over time since index pregnancy more swiftly and in a more comprehensive manner.

Another strength of this study was the availability of vascular results in addition to the clinical measurements and obstetric history in the COPS vascular study cohort.

6.4.3 **Limitations**

Identical biomarker panels were not analysed in both the Generation Scotland and COPS vascular cohorts. It is therefore impossible to know if the results would have been different depending on the age of subjects or using samples prepared differently. A more thorough evaluation would have been to perform identical biomarker studies in both cohorts.
Some of the biomarkers such as IFNγ, IL-2, IL-4, IL-6, IL-10 and TNFα were present in both panels used and were therefore evaluated by two different methods in two different cohorts. Nevertheless, taking into consideration the results of the biomarker studies, it would be useful to evaluate VEGF in the COPS vascular cohort in future.

Study samples were prepared and stored at different times and the protocols between Generation Scotland and the COPS vascular study for sample storage were not identical. Some samples will have been years older than others, and stored in different sites in different freezers from one another. In studies recruiting over even longer periods of time and over several different sites, there will always be inconsistencies even within the same study population, regarding how old the samples are. If there is a large variation in sample time points between subjects in studies recruiting over long periods of time, then sample age should be noted so it can be taken into consideration if necessary. The COPS vascular study cohort was recruited over a shorter period of time than the GS:SFHS and samples were prepared by the same technician and analysed within a much shorter time-frame than GS:SFHS samples. This study was also underpowered to fully assess the potential differences in biomarkers in women with a history of pre-eclampsia and those with normotensive pregnancies.

6.4.4 **Conclusion**

In conclusion, in this chapter the biomarker studies did not entirely agree with the previous literature, indicating not only that characterising specific biomarkers and their roles in cardiovascular disease is complex, but also that different biomarkers may have different influences at different time-points after pregnancy and this should be taken into consideration when evaluating biomarkers for potential future use in this context.
There are not many other studies investigating women at this length of time since index pregnancy, and it is possible that findings more consistent with the findings of this study will present themselves over time. It is also possible that due to the underlying cardiovascular health of the Scottish population, which is worse than other areas of the United Kingdom (444), environment and genetics may play a far greater role than previously thought.

The role of biomarkers not only in the prediction of pre-eclampsia and disease severity, but also in the management of these pregnancies (during the immediate delivery and post-partum period) and their role in the elucidation of cardiovascular disease later in life, is likely to continue to evolve rapidly over the next decade. Rigorous reporting, standardization of techniques for more direct comparison between studies, and adequate sample size and power are just a few of the issues going forward.

It is not realistic to expect that only one or two individual biomarkers will emerge as the answer to predicting cardiovascular disease risk after pre-eclampsia, and that is no longer what is expected in the field. Further research is required to clarify some of the discrepancies in results. A realistic goal for the future might be a biomarker panel, specific for cardiovascular disease risk in women with a history of pre-eclampsia.

7 Discussion

Pre-eclampsia is a serious condition, specific to pregnancy and is traditionally diagnosed as increased blood pressure in the presence of proteinuria with onset >20 weeks gestation. Updated guidelines allow for the diagnosis in the absence of proteinuria if other concerning features are present (15). It remains a main cause of maternal, fetal and neonatal mortality particularly in low- and middle- income countries (58;445). Women with a history of pre-eclampsia are recognised to be at an increased risk of cardiovascular disease later in life and there is ongoing debate as to whether pre-eclampsia itself independently predisposes to this future risk of cardiovascular disease, through processes initiated at the time of pre-eclamptic pregnancy, or whether pre-eclampsia is an indicator of a woman's innate higher risk of future cardiovascular disease. While the studies described in this thesis do not directly address this question, they were designed to further explore the association between cardiovascular disease risk and pre-eclampsia.

The aim of the work presented in this thesis was to address the gap in knowledge regarding mechanisms of the link between pre-eclampsia and cardiovascular disease. I first sought to confirm the relationship between cardiovascular risk and pre-eclampsia in a Scottish cohort, and I then addressed the nature of this relationship through a series of studies designed to assess vascular function, biomarkers and any evidence of cardiac disease on electrocardiogram. A theme throughout the work presented is that the strict phenotype of "pre-eclampsia" was pursued for "cases" in preference to "gestational hypertension", because it is possible that pre-eclampsia and other, less severe forms of hypertensive disorders of pregnancy may have differing underlying pathologies.

7.1 Cardiovascular risk after pre-eclampsia

The first study, described in Chapter 3 used the Generation Scotland: Scottish Family Health Study (GS:SFHS) cohort was able to confirm previous findings in the Scottish population and other countries, that pre-eclampsia does confer future risk of cardiovascular events. In this cohort I found that women with a history of pre-eclampsia were at a 2-fold increased risk of developing cardiovascular disease later in life.

A recent systematic review and meta-analysis of cardiovascular health following pregnancies with pre-eclampsia investigated the future risk of coronary heart disease, heart failure, composite cardiovascular disease, stroke, death due to cardiovascular disease or coronary heart disease and death caused by stroke (446). This was after adjustment for possible confounders such as body mass index, age and diabetes mellitus. It found that pre-eclampsia was associated with a 4-fold increase in the incidence of heart failure and a 2-fold increase in the risk of stroke, coronary heart disease and death from cardiovascular disease or coronary heart disease in the future. The study also found that the risk of stroke, heart failure or death from cardiovascular disease was higher during the 10 years immediately after the affected pregnancy than the following 10 years (446).

7.2 **Electrocardiographic studies**

In order to evaluate the cardiovascular risk in women with a history of pre-eclampsia, I analysed results from the ECGs of women with a history of pre-eclampsia vs normotensive pregnancy in the GS:SFHS. There is evidence of cardiac changes consistent with exposure to a higher blood pressure in women with a history of pre-eclampsia in comparison with normotensive women and in nulliparous vs parous women. There is also evidence of a greater proportion of women with a longer QTc than normal in women who are nulliparous vs parous or women with a history of pre-eclampsia vs normotensive pregnancy. As a result such women may be at greater risk of cardiac arrhythmias and more serious complications.

7.3 **Results of Vascular studies**

To investigate the potential mechanisms underlying the increased cardiovascular risk following pre-eclampsia, I recruited women to the COPS vascular study and assessed clinical and vascular parameters in this cohort of Scottish women.

Blood pressure was found to be elevated in women with a history of pre-eclampsia and heart-rate adjusted AIx, cfPWV and FMD were all statistically significantly different between cases and controls (as was plaque score and presence of plaque). However, only the difference in FMD remained, with pre-eclampsia being an independent predictor of FMD after adjusting for other co-variates. These findings are inconsistent with most other studies described in the literature, but these studies were performed at ~10 years after pre-

eclampsia, which was not as long since index pregnancy as our study. There could now be new evidence of endothelial dysfunction again at an older age. This would possibly be consistent with the findings of Ramsay et al (158) who found decreased endothelial function (microvascular dysfunction) in women 15-25years after pre-eclamptic pregnancy. This group also found similar responses in pregnant women with type 1 diabetes. Some other studies which used the same laser Doppler perfusion imaging found evidence of the opposite pattern in the microvascular response, when FMD found a decreased response (i.e. increased response in microvascular beds was indicative of decreased response in larger vessels). Further research may help to clarify these findings.

7.4 **Biomarker studies**

Given the findings of the vascular studies, it was hypothesised that important potential biomarkers implicated in the picture of endothelial dysfunction years after pre-eclampsia might be revealed. Results of chapter 5 showed that there is no single biomarker to explain the risk of cardiovascular disease following pre-eclampsia. While the results did not reveal any robust biomarker that is associated with a history of pre-eclampsia, this does not mean that there is no biomarker that could predict cardiovascular events in these women. However, I did not specifically address the question of which biomarker predicts cardiovascular events, nor was my study designed to do so. I did not study women who had developed cardiovascular disease already, after pre-eclampsia, but rather I compared women who developed pre-eclampsia during their remote pregnancy with women who had experienced normotensive remote pregnancies. This was done with a view to comparing which biomarkers may be useful in determining some of the differences observed in the vascular and record-linkage studies between these two groups of women.

The finding of a raised VEGF level in women with a history of pre-eclampsia and lower FMD, as seen in chapter 4, is a pattern that has been seen before, out-with pre-eclampsia research. For example, in a study examining endothelial dysfunction, abnormal thrombogenesis and abnormal angiogenesis in hypertensive men (443) 76 men (aged 40-60yrs) with a diagnosis of hypertension were compared with 48 normotensive controls. FMD was significantly lower in the hypertensive group and VEGF levels significantly higher along with tissue factor (TF) and von Willebrand factor (vWF). After intensified blood pressure and hypercholesterolaemia treatment, the levels of TF, vWF and VEGF all decreased and FMD increased. Considering that in chapter 4 women with a remote history

of pre-eclampsia were found to have a higher blood pressure than controls, it is possible that there are similar underlying mechanisms involved.

Another complicating factor to consider is the heterogeneity of certain biomarkers across other diseases and conditions (not just specific to pre-eclampsia or cardiovascular disease). Also, the expectation of patients and health clinics must take into account the fact that risks will change over time and where a patient is on the cardiovascular disease continuum will change. While early changes may not be picked up on one appointment, how long should the interval be between this point in time and screening again? Five years may be sufficient time for a patient to develop and die from more serious sequelae. Other considerations would be the clinical utility of the biomarker test and whether it would be cost-effective and timely to deliver.

7.5 **Limitations**

The studies were underpowered to investigate timing of onset of pre-eclampsia or investigate recurrent pre-eclampsia. Further exploration of the timing of pre-eclampsia could be important when one considers that women with early onset pre-eclampsia at less than 32 weeks (compared with ≥37 weeks) are at a 20-fold increased maternal mortality risk (22;447).

I also did not adjust for menopause as a co-variate. The study would have been underpowered to analyse women according to menopausal status, and due to the fact that the menopause evolves over a period of time and is not a suddenly apparent finding, and considering that our data was questionnaire based and menopausal status was not assessed by any biochemical measurement, the data would not have been reliable. However, menopause does have effects on the blood pressure. Systolic blood pressure is increased, most likely secondary to increased angiotensin II receptor expression, withdrawal of vasodilator effects of endogenous oestrogen, reduced endothelial nitric oxide production, increased salt sensitivity and arterial stiffness (448;449). An isolated increase in systolic blood pressure is a predictor of later cardiovascular disease in both men and women (449).

Similarly, I did not adjust for type of contraception, and oral contraceptive use has been associated with increases in blood pressure and risk of cardiovascular events (448). Such

increases are thought likely secondary to renin-angiotensin aldosterone activation, salt and water retention and increased arterial stiffness.

Another factor which was not adjusted for was socio-economic status. Low maternal socio-economic status is a strong risk factor for pre-eclampsia (450) and higher incidence of cardiovascular disease has been found in more deprived areas (451). Further consideration of socio-economic status is warranted, especially considering the inequalities in health and levels of socio-economic deprivation in Glasgow, the city from which the highest number of study participants were recruited. The prevalence of cardiovascular disease in Glasgow is higher in comparison with other UK cities, and five out of the ten local authorities with the highest cardiovascular disease mortality rates in the UK are found in Scotland (444). Glasgow had the highest cardiovascular disease death rates at under 75 years (144/100,000 population) and at all ages (400/100,000) in a recent study (444).

7.6 **Future directions**

Improving knowledge about the consequences of pre-eclampsia, not only amongst patients, but in the wider medical community is warranted. The legacy of pre-eclampsia is important not only for women, but also their children, as offspring are also at risk of cardiovascular disease. Previous studies have revealed sub-optimal knowledge amongst health care physicians (452;453), but with pre-eclampsia featuring in the American Heart Association 2011 guidelines for the prevention of cardiovascular disease in women (89), it is hoped that this will change. Nevertheless, efforts to educate patients and physicians should focus on appropriate post-partum information about the consequences of pre-eclampsia, as mentioned in the NICE guidelines for hypertension in pregnancy (2). Communication and swift easy transfer of information between primary and secondary care would facilitate thorough and comprehensive follow-up for these women. General Practitioners are tasked with the routine care and follow-up of these women in the community and require adequate resources for monitoring. They have long-term responsibility for these patients and therefore require the appropriate information in a timely fashion.

Further education about the prediction and prevention of pre-eclampsia will also be of benefit. For example, an increase in body mass index between pregnancies and obesity increases the risk of recurrent pre-eclampsia (454). The NICE guideline advises women who have had pre-eclampsia to try to achieve and maintain a body mass index in the

healthy range prior to a subsequent pregnancy (2). Furthermore, there is evidence that chronic hypertension after hypertensive disorders of pregnancy might be reduced if a healthy lifestyle is adhered to, in particular a healthy weight (455).

The dilemma of when and how often to follow-up these women is a matter of ongoing debate. Whilst pre-eclampsia increases cardiovascular risk overall, those with recurrent episodes of pre-eclampsia are at a higher level of risk and need more attention. According to national guidelines in both the United Kingdom and the United States (2;89;90) women should be followed up after a pre-eclamptic pregnancy, however, there is a lack of evidence concerning the extent of monitoring and length of the follow-up period itself (446). Ideally follow-up should be cost-effective and should not have negative effects. Further research into the precise methods and length of follow-up should be explored.

Further research is required to advance our understanding of the mechanisms behind the link between pre-eclampsia and future cardiovascular disease. In order to unravel the complexities of hypertensive disorders of pregnancy, and the likely subtle differences between different types of disorder (e.g. term vs pre-term pre-eclampsia), collaboration will play an increasingly important role. The generation of large studies and large datasets will reduce the likelihood of underpowered studies. Longitudinal studies including pre-pregnancy time-points will also be very important. Establishing which risk factors were present prior to pregnancy and to what extent (e.g. degree of endothelial dysfunction) will provide a definitive answer how much pre-eclampsia and hypertensive disorders of pregnancy contribute to cardiovascular risk after such pregnancies. It is likely that a woman's pre-existing, pre-pregnancy constitution, in combination with factors relating to pre-eclampsia itself may be responsible for future cardiovascular disease overall.

Maximisation of resources by standardisation of study design is another route by which large pieces of information could be utilised efficiently. A standardised outcome set for studies of hypertensive disorders of pregnancy is being created by the Core Outcome Measures in Effectiveness Trials (COMET) (58). The Global Pregnancy Collaboration (CoLab) has already established standardised study design for use in pre-eclampsia research (456).

7.7 **Conclusion**

Despite the large amount of research which has already been done to investigate the causes of cardiovascular disease in women with a remote history of pre-eclampsia, the precise mechanisms responsible for this relationship have yet to be confirmed.

The studies described in this thesis have not identified one particular component which explains the increased cardiovascular risk in this group of women, nor have they clarified the precise nature of the association between pre-eclampsia and cardiovascular disease. It remains unclear whether pre-eclampsia itself is responsible for the increase in cardiovascular risk or whether pre-eclampsia is an indication of an enhanced cardiovascular risk which pre-dated the pregnancy. However, these results are not unexpected due to the complex nature of the condition. There will be many factors which contribute to the evolution of pre-eclampsia, and it is possible that the increased cardiovascular risk later in life will also be composed of many different elements. Maternal constitution is likely to be an important factor in the development of pre-eclampsia and later cardiovascular disease, as evidenced by data from the HUNT study (103).

Perhaps more thorough assessment and investigation of these women could yield more informative results. For example, the studies included in this thesis did not involve performing echocardiography or MRI imaging. Future studies should focus on assessing an even greater range of modalities in investigating the relationship between pre-eclampsia and cardiovascular disease. Differing follow-up periods, consideration of new studies re-visiting investigation in existing study cohorts and the possibility of merging study cohorts or wider collaboration should also be considered. Follow-up periods could be important in further determination of the pathophysiology of cardiovascular risk after pre-eclampsia. For example, it would have been interesting to assess findings at later time-points in studies of flow-mediated dilatation which detected endothelial dysfunction at 1 year but normalisation of these changes by 10 years. It is not possible to know whether normalisation of endothelial dysfunction remained in these women at 15 years and 20 years post-partum.

As the causes of pre-eclampsia and subtle differences in pathophysiology between the different types of pre-eclampsia (e.g. early-onset vs late-onset) and other hypertensive disorders of pregnancy are further elucidated, mechanisms behind the relationship between

pre-eclampsia and cardiovascular disease later in life may be further explored. Improvements in the understanding of this condition will help identify which specific women are at higher risk of cardiovascular disease, thus allowing more targeted interventions in a timely manner. They will also aid in the discovery of novel therapies which may be of great benefit to these women.

Appendix1: COPS study invitation letter for GS:SFHS participants

NHS Greater Glasgow and Clyde

University of Glasgow

BHF Glasgow Cardiovascular Research Centre
Cardiovascular Consequences of Preeclampsia (COPS) Study

Dear Mrs Specific Name,

A few years ago you took part in the Generation Scotland: Scottish Family Health (GS:SFHS) study. This important project has already provided a huge amount of information about the genetic basis behind some of the common diseases affecting families in Scotland.

We would now like to invite you to participate in a related research project we are undertaking at Glasgow University. The aim of the study is to find out more about the relationship between pre-eclampsia, a condition where pregnant women develop high blood pressure, and cardiovascular diseases. We are inviting women who had pre-eclampsia during their pregnancies 10-30 years ago, and women who had normal blood pressure during their pregnancies. **In particular we hope, as a result of the research project, to be able to understand why women who suffer from pre-eclampsia appear to be at higher risk of high blood pressure, heart disease and kidney disease in later life.**

Please take the time to read the enclosed patient information leaflet and consent form, and if you have any questions please contact us. The study visit, which lasts around 90 minutes, will normally be during working hours (Monday to Friday 9am-5pm,) but if you are only able to attend outwith these times we will do our best to accommodate this. We will provide taxis to and from the study visit if required, as well as refreshments.

If you are interested in taking part, or have any questions, please contact Joanne Flynn, Research Nurse on 0141 232 9515 or 0141 330 4565, or email joanne.flynn@ggc.scot.nhs.uk

We look forward to seeing you,

Dr Catriona Brown, Dr David Carty, Dr Christian Delles, Professor Anna Dominiczak
Cardiovascular Consequences of Preeclampsia Study Team

BHF Glasgow Cardiovascular Research Centre, University of Glasgow
126 University Place, Glasgow G12 8TA, Scotland, UK
Telephone: +44 (141) 330-4558 *Fax:* +44 (141) 330-6997

Version GS.1.3 – 21/08/2013

Appendix 2: COPS study invitation letter for clinic participants

BHF Glasgow Cardiovascular Research Centre
Cardiovascular Consequences of
Preeclampsia Study Team

University of Glasgow

NHS Greater Glasgow and Clyde

Dear Madam,

We would like invite you to participate in a research project we are carrying out at Glasgow University. The aim of the research project is to find out more information about the relationship between preeclampsia, a condition where pregnant women develop high blood pressure, and cardiovascular diseases. In particular we hope, as a result of the research project, to be able to understand why women who suffer from preeclampsia appear to be at increased risk of high blood pressure, heart disease and kidney disease in later life.

We are inviting women who attend blood pressure clinics in Glasgow to participate in this research. We are inviting both women who had preeclampsia during their pregnancies 1-30 years ago, and women who had normal blood pressure during their pregnancies. Please take the time to read the enclosed Patient Information Leaflet and consent form, and if you would be interested in taking part in the study, or have any questions about the study please contact us.

If you are interested in participating please contact Ms Joanne Flynn, Research Nurse on 0141 232 9515 or alternatively email on joanne.flynn@ggc.scot.nhs.uk

Best wishes,

Dr Christian Delles, Dr David Carty, Dr Marie Freel, Prof Anna Dominiczak

Cardiovascular Consequences of Preeclampsia Study Team

BHF Glasgow Cardiovascular Research Centre, University of Glasgow
126 University Place, Glasgow G12 8TA, Scotland, UK
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Version 1.2 – 15/05/2013

Appendix 3: COPS study invitation letter for PIP participants

NHS Greater Glasgow and Clyde

University of Glasgow

BHF Glasgow Cardiovascular Research Centre
Cardiovascular Consequences of Preeclampsia (COPS) Study

Dear Mrs Specific Name,

A few years ago you took part in the Proteomics in Preeclampsia (PIP) study. This important study has already provided a large amount of information about preeclampsia.

We would now like to invite you to participate in a related research project we are undertaking at Glasgow University. The aim of the study is to find out more about the relationship between pre-eclampsia, a condition where pregnant women develop high blood pressure, and cardiovascular diseases. We are inviting women who had pre-eclampsia during their pregnancies 1-10 years ago, and women who had normal blood pressure during their pregnancies. **In particular we hope, as a result of the research project, to be able to understand why women who suffer from pre-eclampsia appear to be at higher risk of high blood pressure, heart disease and kidney disease in later life.**

Please take the time to read the enclosed patient information leaflet and consent form, and if you have any questions please contact us. The study visit, which lasts around 90 minutes, will normally be during working hours (Monday to Friday 9am-5pm,) but if you are only able to attend outwith these times we will do our best to accommodate this. We will provide taxis to and from the study visit if required, as well as refreshments.

If you are interested in taking part, or have any questions, please contact Joanne Flynn, Research Nurse on 0141 232 9515 or 0141 330 4565, or email joanne.flynn@ggc.scot.nhs.uk

We look forward to seeing you,

Dr Catriona Brown, Dr David Carty, Dr Christian Delles, Professor Anna Dominiczak
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Version pip.1.3 – 21/08/2013

Appendix 4: COPS patient information sheet for GS:SFHS participants

COPS; GS

Version 1.3. 19/3/2013

Contact for further information:
Dr Catriona Brown
BHF Glasgow Cardiovascular Research Centre
University of Glasgow
0141 330 ****

University of Glasgow

NHS Greater Glasgow and Clyde

Patient information: Cardiovascular Consequences of Preeclampsia study

You are being invited to take part in a research study (Cardiovascular Consequences of Preeclampsia study.) Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. If there is anything that is not clear, or if you would like more information please contact us.

What is the purpose of the study?

Pre-eclampsia is a condition in which pregnant women can develop high blood pressure. It affects approximately 5% of all pregnancies. Although the blood pressure returns to normal after pregnancy, there is now increasing evidence that women who suffer from preeclampsia are at a small, but significantly increased risk of future development of high blood pressure, heart disease, kidney disease and stroke. The reasons for this are not well understood. In this study we aim to find out more about the relationship between preeclampsia and future cardiovascular diseases. It is hoped that this information will help us to work out why women who develop preeclampsia appear to be at increased risk.

Why have I been chosen to take part?

You have been chosen because you previously participated in the Generation Scotland: Scottish Family Health Study. We are inviting women who took part in that study who had preeclampsia during their pregnancy 10-30 years ago, and women who had "normal" uncomplicated pregnancies 10-30 years ago.

Do I have to take part?

No. It is up to you to decide whether or not to take part: participation is completely voluntary. If you do decide to take part you will be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason.

What will be involved if I decide to take part?

If you decide to take part, we will invite you to come to our research centre for a 90 minutes appointment. We will carry out the following tests:

- We would like to measure your blood pressure, using a machine similar to ones used at your GP.
- We would like to take a sample of blood (about 3 tablespoonfuls) and a sample of urine, which will be stored in our lab and may be analysed at a later date.
- We would like to examine the arteries at your radial artery (wrist), carotid artery (neck) and femoral artery (groin) with a pencil-like probe. This test takes 5-10 minutes and gives us useful information about blood vessel function.
- We would like to attach small probes to your index fingers, which measure the blood flow. We will then inflate a blood pressure cuff for 5 minutes, and then deflate it and continue to monitor the blood flow to the fingers. This test takes about 20 minutes, and can give us useful information about the endothelium, which is the inner layer of blood vessels.
- We would like to examine the thickness of the wall of your carotid artery. This is the large artery in your neck providing blood to the brain. We know that this wall thickness is related to the cardiovascular risk profile of an individual patient. This examination is performed with an ultrasound probe which is placed on your carotid artery for a few minutes.
- We would like to use the ultrasound scanner to look at the large artery in the upper arm (brachial artery). We will inflate a blood pressure cuff around the forearm for 5 minutes and we will record the width of the artery for a few minutes after release of cuff pressure. The test takes about 15 minutes.

What are the risks of taking part in this research?

The amount of blood taken for this research does not place you at any risk. The other tests are "non-invasive," that is that probes are only attached to your skin. Measurement of blood flow in your finger may lead to some numbness in your arm during the test, which will disappear when the cuff is deflated. A small bruise at your forearm may result from the cuff but will disappear within one or two days.

What are the benefits of taking part?

There is no direct benefit for you from taking part in this study. However, the information we get from this study may help us in the future to understand the relationship between preeclampsia and cardiovascular disease in later life. We will feed back routine results to you that are currently used to assess risk of heart disease, e.g. blood pressure, and if you wish we can also forward copies to your GP. If, in the unlikely event that we feel you require more urgent treatment, we may refer you to the local hospital.

Will my taking part in this study be kept confidential?

Your personal information will be kept on a file and stored in a secure place at the BHF Glasgow Cardiovascular Research Centre. All samples and test results will be labelled with a code and not with any personal details so that all analyses will be carried out anonymously. All information which is collected about you during the course of the research will be kept strictly confidential. We may continue to collect information about your health for up to 10 years. Any information about you which leaves the Clinical Investigation Unit will have your name and address removed so that you cannot be recognised from it.

What will happen to any samples I give?

You will donate blood and urine samples for research purposes. Some examinations on these samples will be done straight away. Other examinations will be done at a later stage when we collect more samples from other patients. We will also store some of the samples in our laboratory, so that we can perform additional tests in the future if required. The samples are treated as "gift"; this means you will not be entitled to any future financial reimbursement related to this study and related research.

What will happen to the results of the research study?

The results of the research study will be stored on a computer database and are likely to be published in medical journals. Reports or publications resulting from the study will not contain any personal details. The research doctor will provide a copy of the results on request.

What happen if something goes wrong?

In the unlikely event that something goes wrong, please contact the chief investigator, Dr Christian Delles, who will try to resolve any issues. If this is not possible, then you should contact the NHS Greater Glasgow and Clyde complaints procedure on 0141 201 4500.

Who is organising and funding the research?

The research is organised by the Institute of Cardiovascular and Medical Sciences, University of Glasgow, in collaboration with the Generation Scotland: Scottish Family Health Study. The study is funded by the Chief Scientist's Office of the Scottish Government and researchers will not receive any payment for conducting this research. The study is sponsored by NHS Greater Glasgow and Clyde.

Contact for Further Information

Should you have any further questions about the study please feel free to call Dr Catriona Brown, Dr David Carty or Dr Christian Delles on 0141 330 ****. To speak to someone independent for any more general enquiries about research please contact Professor Alan Jardine at the University of Glasgow Medical School on 0141 330 2705. Thank you for taking the time to read this information sheet.

Appendix 5: COPS patient information sheet for clinic participants

COPS; Clinic

Version 1.4. 15/5/2013

Contact for further information:
Dr Catriona Brown
BHF Glasgow Cardiovascular Research Centre
University of Glasgow
0141 330 5189

University of Glasgow

NHS Greater Glasgow and Clyde

Patient information: Cardiovascular Consequences of Preeclampsia study

You are being invited to take part in a research study (Cardiovascular Consequences of Preeclampsia study.) Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. If there is anything that is not clear, or if you would like more information please contact us.

What is the purpose of the study?

Pre-eclampsia is a condition in which pregnant women can develop high blood pressure. It affects approximately 5% of all pregnancies. Although the blood pressure returns to normal after pregnancy, there is now increasing evidence that women who suffer from preeclampsia are at a small, but significantly increased risk of future development of high blood pressure, heart disease, kidney disease and stroke. The reasons for this are not well understood. In this study we aim to find out more about the relationship between preeclampsia and future cardiovascular diseases. It is hoped that this information will help us to work out why women who develop preeclampsia appear to be at increased risk.

Why have I been chosen to take part?

You have been chosen because you have been attending a blood pressure clinic at a Glasgow Hospital. We are inviting women who attend blood pressure clinics who had preeclampsia during their pregnancies 1-30 years ago, and women who had "normal" uncomplicated pregnancies 1-30 years ago.

Do I have to take part?

No. It is up to you to decide whether or not to take part: participation is completely voluntary. If you do decide to take part you will be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason.

What will be involved if I decide to take part?

If you decide to take part, we will invite you to come to our research centre for a 90 minutes appointment. We will carry out the following tests:

- We would like to measure your blood pressure, using a machine similar to ones used at your GP.
- We would like to take a sample of blood (about 3 tablespoonfuls) and a sample of urine, which will be stored in our lab and may be analysed at a later date.
- We would like to examine the arteries at your radial artery (wrist), carotid artery (neck) and femoral artery (groin) with a pencil-like probe. This test takes 5-10 minutes and gives us useful information about blood vessel function.
- We would like to attach small probes to your index fingers, which measure the blood flow. We will then inflate a blood pressure cuff for 5 minutes, and then deflate it and continue to monitor the blood flow to the fingers. This test takes about 20 minutes, and can give us useful information about the endothelium, which is the inner layer of blood vessels.
- We would like to examine the thickness of the wall of your carotid artery. This is the large artery in your neck providing blood to the brain. We know that this wall thickness is related to the cardiovascular risk profile of an individual patient. This examination is performed with an ultrasound probe which is placed on your carotid artery for a few minutes.
- We would like to use the ultrasound scanner to look at the large artery in the upper arm (brachial artery). We will inflate a blood pressure cuff around the forearm for 5 minutes and we will record the width of the artery for a few minutes after release of cuff pressure. The test takes about 15 minutes.

What are the risks of taking part in this research?

The amount of blood taken for this research does not place you at any risk. The other tests are "non-invasive," that is that probes are only attached to your skin. Measurement of blood flow in your finger may lead to some numbness in your arm during the test, which will disappear when the cuff is deflated. A small bruise at your forearm may result from the cuff but will disappear within one or two days.

What are the benefits of taking part?

There is no direct benefit for you from taking part in this study. However, the information we get from this study may help us in the future to understand the relationship between preeclampsia and cardiovascular disease in later life. We will feed back routine results to you that are currently used to assess risk of heart disease, e.g. blood pressure, and if you wish we can also forward copies to your GP. If, in the unlikely event that we feel you require more urgent treatment, we may refer you to the local hospital.

Will my taking part in this study be kept confidential?

Your personal information will be kept on a file and stored in a secure place at the BHF Glasgow Cardiovascular Research Centre. All samples and test results will be labelled with a code and not with any personal details so that all analyses will be carried out anonymously. All information which is collected about you during the course of the research will be kept strictly confidential. We may continue to collect information about your health for up to 10 years. Any information about you which leaves the Clinical Investigation Unit will have your name and address removed so that you cannot be recognised from it.

What will happen to any samples I give?

You will donate blood and urine samples for research purposes. Some examinations on these samples will be done straight away. Other examinations will be done at a later stage when we collect more samples from other patients. We will also store some of the samples in our laboratory, so that we can perform additional tests in the future if required. The samples are treated as "gift"; this means you will not be entitled to any future financial reimbursement related to this study and related research.

What will happen to the results of the research study?

The results of the research study will be stored on a computer database and are likely to be published in medical journals. Reports or publications resulting from the study will not contain any personal details. The research doctor will provide a copy of the results on request.

What happen if something goes wrong?

In the unlikely event that something goes wrong, please contact the chief investigator, Dr Christian Delles, who will try to resolve any issues. If this is not possible, then you should contact the NHS Greater Glasgow and Clyde complaints procedure on 0141 201 4500.

Who is organising and funding the research?

The research is organised by the Institute of Cardiovascular and Medical Sciences, University of Glasgow, in collaboration with the Generation Scotland: Scottish Family Health Study. The study is funded by the Chief Scientist's Office of the Scottish Government and researchers will not receive any payment for conducting this research. The study is sponsored by NHS Greater Glasgow and Clyde.

Contact for Further Information

Should you have any further questions about the study please feel free to call Dr Catriona Brown, Dr David Carty or Dr Christian Delles on 0141 330 2749. To speak to someone independent for any more general enquiries about research please contact Professor Alan Jardine at the University of Glasgow Medical School on 0141 330 2705. Thank you for taking the time to read this information sheet.

Appendix 6: COPS patient information sheet for PIP participants

COPS; PIP Version 1.3. 19/03/2013

Contact for further information:
Dr Catriona Brown
BHF Glasgow Cardiovascular Research Centre
University of Glasgow
0141 330 ****

University of Glasgow

NHS Greater Glasgow and Clyde

Patient information: Cardiovascular Consequences of Preeclampsia study

You are being invited to take part in a research study (Cardiovascular Consequences of Preeclampsia study.) Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. If there is anything that is not clear, or if you would like more information please contact us.

What is the purpose of the study?

Pre-eclampsia is a condition in which pregnant women can develop high blood pressure. It affects approximately 5% of all pregnancies. Although the blood pressure returns to normal after pregnancy, there is now increasing evidence that women who suffer from preeclampsia are at a small, but significantly increased risk of future development of high blood pressure, heart disease, kidney disease and stroke. The reasons for this are not well understood. In this study we aim to find out more about the relationship between preeclampsia and future cardiovascular diseases. It is hoped that this information will help us to work out why women who develop preeclampsia appear to be at increased risk.

Why have I been chosen to take part?

You have been chosen because you previously participated in the "Proteomics in Preeclampsia (PIP) study. We are inviting women who took part in that study who had preeclampsia during their pregnancy 2-5 years ago, and women who had "normal" uncomplicated pregnancies 2-5 years ago.

Do I have to take part?

No. It is up to you to decide whether or not to take part: participation is completely voluntary. If you do decide to take part you will be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason.

What will be involved if I decide to take part?

If you decide to take part, we will invite you to come to our research centre for a 90 minutes appointment. We will carry out the following tests:

- We would like to measure your blood pressure, using a machine similar to ones used at your GP.
- We would like to take a sample of blood (about 3 tablespoonfuls) and a sample of urine, which will be stored in our lab and may be analysed at a later date.
- We would like to examine the arteries at your radial artery (wrist), carotid artery (neck) and femoral artery (groin) with a pencil-like probe. This test takes 5-10 minutes and gives us useful information about blood vessel function.
- We would like to attach small probes to your index fingers, which measure the blood flow. We will then inflate a blood pressure cuff for 5 minutes, and then deflate it and continue to monitor the blood flow to the fingers. This test takes about 20 minutes, and can give us useful information about the endothelium, which is the inner layer of blood vessels.
- We would like to examine the thickness of the wall of your carotid artery. This is the large artery in your neck providing blood to the brain. We know that this wall thickness is related to the cardiovascular risk profile of an individual patient. This examination is performed with an ultrasound probe which is placed on your carotid artery for a few minutes.
- We would like to use the ultrasound scanner to look at the large artery in the upper arm (brachial artery). We will inflate a blood pressure cuff around the forearm for 5 minutes and we will record the width of the artery for a few minutes after release of cuff pressure. The test takes about 15 minutes.

What are the risks of taking part in this research?

The amount of blood taken for this research does not place you at any risk. The other tests are "non-invasive," that is that probes are only attached to your skin. Measurement of blood flow in your finger may lead to some numbness in your arm during the test, which will disappear when the cuff is deflated. A small bruise at your forearm may result from the cuff but will disappear within one or two days.

What are the benefits of taking part?

There is no direct benefit for you from taking part in this study. However, the information we get from this study may help us in the future to understand the relationship between preeclampsia and cardiovascular disease in later life. We will feed back routine results to you that are currently used to assess risk of heart disease, e.g. blood pressure, and if you wish we can also forward copies to your GP. If, in the unlikely event that we feel you require more urgent treatment, we may refer you to the local hospital.

Will my taking part in this study be kept confidential?

Your personal information will be kept on a file and stored in a secure place at the BHF Glasgow Cardiovascular Research Centre. All samples and test results will be labelled with a code and not with any personal details so that all analyses will be carried out anonymously. All information which is collected about you during the course of the research will be kept strictly confidential. We may continue to collect information about your health for up to 10 years. Any information about you which leaves the Clinical Investigation Unit will have your name and address removed so that you cannot be recognised from it.

What will happen to any samples I give?

You will donate blood and urine samples for research purposes. Some examinations on these samples will be done straight away. Other examinations will be done at a later stage when we collect more samples from other patients. We will also store some of the samples in our laboratory, so that we can perform additional tests in the future if required. The samples are treated as "gift"; this means you will not be entitled to any future financial reimbursement related to this study and related research.

What will happen to the results of the research study?

The results of the research study will be stored on a computer database and are likely to be published in medical journals. Reports or publications resulting from the study will not contain any personal details. The research doctor will provide a copy of the results on request.

What happen if something goes wrong?

In the unlikely event that something goes wrong, please contact the chief investigator, Dr Christian Delles, who will try to resolve any issues. If this is not possible, then you should contact the NHS Greater Glasgow and Clyde complaints procedure on 0141 201 4500.

Who is organising and funding the research?

The research is organised by the Institute of Cardiovascular and Medical Sciences, University of Glasgow, in collaboration with the Generation Scotland: Scottish Family Health Study. The study is funded by the Chief Scientist's Office of the Scottish Government and researchers will not receive any payment for conducting this research. The study is sponsored by NHS Greater Glasgow and Clyde.

Contact for Further Information

Should you have any further questions about the study please feel free to call Dr Catriona Brown, Dr David Carty or Dr Christian Delles on 0141 330 ****. To speak to someone independent for any more general enquiries about research please contact Professor Alan Jardine at the University of Glasgow Medical School on 0141 330 2705. Thank you for taking the time to read this information sheet.

Appendix 7: COPS information letter for GP

BHF Glasgow Cardiovascular Research Centre
Cardiovascular Consequences of Preeclampsia Study Team

University of Glasgow

NHS Greater Glasgow and Clyde

###Name###
###Address Line 1###
###Address Line 2###
###Address Line 3###
###Address Line 4###

###Post Code###

Dear Doctor ###Name###,

Re: ###Patient Name###, ###DOB###
Cardiovascular Consequences of Preeclampsia Study

Your patient has agreed to participate in a research study. This letter is to give you some information about the study.

We know from epidemiological studies that women with a history of preeclampsia have an increased risk of future development of hypertension, stroke, renal disease and coronary artery disease. This relationship is poorly understood, and we aim to find out more about the mechanisms behind this increased risk. We are recruiting women who previously participated in the Generation Scotland : Scottish Family Health Study, women who participated in the Proteomics in Preeclampsia (PIP) study, and women who attend blood pressure and cardiovascular risk factor clinics in Glasgow. We are recruiting both women who suffered from preeclampsia in the past and women who had normotensive pregnancies. We will examine for signs of vascular damage, both using clinical tests and searching for novel biomarkers that may help to define their longer term cardiovascular risk.

None of the study-related tests and analyses are currently established for clinical purposes. We will therefore not routinely report results back to you. However, should you wish further information about the study, please contact Dr Christian Delles, Dr David Carty or Prof. Anna Dominiczak on 0141 330 4558.

Yours sincerely,

Dr Christian Delles, Dr David Carty, Dr Marie Freel, Prof Anna Dominiczak
Cardiovascular Consequences of Preeclampsia study team.

BHF Glasgow Cardiovascular Research Centre, University of Glasgow
126 University Place, Glasgow G12 8TA, Scotland, UK
Telephone: +44 (141) 330-4558 *Fax:* +44 (141) 330-6997

Version 1.1 – 06/11/12

Appendix 8: COPS thank you letter for participants

BHF Glasgow Cardiovascular Research Centre
Cardiovascular Consequences of
Preeclampsia Study Team

University of Glasgow

NHS Greater Glasgow and Clyde

Dear Madam,

We would like to thank you for participating in our research project. We hope, as a result of this study, to be better able to understand why women who suffer from preeclampsia appear to be at increased risk of high blood pressure, heart disease and kidney disease in later life.

The majority of the tests that we have carried out are for research purposes, and the information from them is of no direct clinical benefit to you. As such we do not routinely feed back results from these tests to you or your General Practitioner.

The results of the study will be published in medical journals, and information about any research papers resulting from the study will be available on the Generation Scotland website www.generationscotland.org

Thank you once again for your participation

Best wishes,

Dr Christian Delles, Dr David Carty, Dr Marie Freel, Prof Anna Dominiczak

Cardiovascular Consequences of Preeclampsia Study Team

BHF Glasgow Cardiovascular Research Centre, University of Glasgow
126 University Place, Glasgow G12 8TA, Scotland, UK
Telephone: +44 (141) 330-4558 *Fax:* +44 (141) 330-6997

Version 1.1 – 06/11/2012

Appendix 9: Consent form for COPS study

NHS Greater Glasgow and Clyde

University of Glasgow

CONSENT FORM

Cardiovascular Consequences of Preeclampsia Study

Name of Researcher:
Dr C Delles
Version : Clinic V1.0
Date: 22/08/14

Patient Identification Code for this Study: | | - | | | | | | - | |

I agree to take part in the above project.

	Please initial box
• I confirm that I have read and understand the information sheet version _____ dated _____for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐
• I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	☐
• I understand that sections of any of my medical and obstetric notes and data collected during the study may be looked at by members of the research team, where it is relevant to my taking part in this research, or by responsible persons from NHS Greater Glasgow and Clyde. I give permission for these individuals to have access to my records.	☐
• I agree to donate samples of blood and urine, for the purposes of the research project and to undergo tests to look at the function of my blood vessels. I understand that samples will be examined for expression of genes and proteins that may be related to preeclampsia, and may be stored for analysis in future studies.	☐
• I agree that my GP may be informed of my taking part in this study and, if relevant, informed of results from the examinations.	☐
• I agree that the research team may continue to collect information about my health for up to 10 years and may contact me about my health, where relevant to the study.	☐

_____	_____	_____
Name of Patient	Date	Signature
_____	_____	_____
Name of Person taking consent	Date	Signature

BHF Glasgow Cardiovascular Research Centre, University of Glasgow
126 University Place, Glasgow G12 8TA, Scotland, UK
Telephone: +44 (141) 330-4558 *Fax:* +44 (141) 330-6997

Appendix 10: COPS study questionnaire

University of Glasgow | BHF Glasgow Cardiovascular Research Centre

NHS Greater Glasgow and Clyde

STUDY CODE ..... **INITIALS**..... **DATE:** ...../...../.....

COPS STUDY QUESTIONNAIRE

1. ETHNICITY Caucasian ☐ Non-Caucasian ☐ .....

2. GENERATION SCOTLAND participant **Y / N**

3. OBSTETRIC AND GYNAECOLOGICAL HISTORY

a)Pregnancies (list in chronological order, first to last)

(include miscarriages/stillbirths in the correct chronological order and gestation if possible)

Year of pregnancy (in yrs)	Pre eclampsia Y / N	Gest^{n} at Dx	Gest^{n} at delivery	Mode of delivery	Birth weight	Mother and baby health after birth?	BP meds reqd during/after pregnancy	Hospital of birth

b)Gynaecological

Age at menarche .....yrs

Date of LMP ...../...../.....

Usual cycle length (in days).....

Hormonal Contraception **Y / N**

(If Y, list preparations and timings):.....

Menopause **Y / N**

(If Y, been on HRT? List type/timings):.....

4.MEDICAL HISTORY	**Y / N**	**YEAR OF ONSET**
Hypertension	.....	.....
Ischaemic Heart Disease	.....	.....
Stroke	.....	.....
Diabetes	.....	.....
Renal disease	.....	.....
Asthma	.....	.....
COPD	.....	.....
Depression	.....	.....
Autoimmune disease	.....	.....
Epilepsy	.....	.....
Cancer	.....	.....
Other illnesses (please specify)	.....	.....

Nov 2013 CB Version 1.3

University of Glasgow | BHF Glasgow Cardiovascular Research Centre

NHS Greater Glasgow and Clyde

5.DRUG HISTORY

Current medications:

Name	Dose	Time	Name	Dose	Time
Allergies:					

6.FAMILY HISTORY (FHx of conditions listed in No.4, and in addition preeclampsia)

.....
.....
.....
.....
.....
.....
.....
.....

7.SOCIAL HISTORY

Smoking:	**Y / N**	
Current	.....	If Y how many?.....
Ex-smoker	.....	If Y when stopped?.....
		How many before stopped?.....
Never smoker	.....	

Alcohol:	**Y / N**
Nil	.....
<14 units/wk	.....
14-21 units/wk	.....
>21 units/wk	.....

8.STUDY PATIENT MEASUREMENTS/INITIAL TESTS

Age .....yrs
Height .....m
Weight .....kg
BMI .....kg/m^2
BP .....mmHg .....mmHg .....mmHg
HR .....bpm

	Y/N
ECG carried out	.....
Urine sample	.....
Bloods taken	..... (2 purple tops, 1 green top, 2 gold tops, 1 blue TEMPUS tube)

Appendix 11: COPS Ethical approval

WoSRES
West of Scotland Research Ethics Service

NHS
Greater Glasgow and Clyde

West of Scotland REC 3
Ground Floor – The Tennent Institute
Western Infirmary
38 Church Street
Glasgow G11 6NT
www.nhsggc.org.uk

Dr Christian Delles
BHF Glasgow Cardiovascular Research Centre
126 University Place
Glasgow
G12 8TA

Date 19th December 2012
Your Ref
Our Ref
Direct line 0141 211 2123
Fax 0141 211 1847
E-mail Liz.Jamieson@ggc.scot.nhs.uk

Dear Dr Delles

Study title: **Cardiovascular consequences of preeclampsia study**
REC reference: **12/WS/0306**
Protocol number: **1.1**
IRAS project ID: **108706**

The Research Ethics Committee reviewed the above application at the meeting held on 13 December 2012. The Committee would like to thank Dr Carty for attending on your behalf.

We plan to publish your research summary wording for the above study on the NRES website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the Co-ordinator Mrs Liz Jamieson, Liz.Jamieson@ggc.scot.nhs.uk.

Ethical opinion

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Discussion

1) The Committee agreed that the recruitment process was unclear. Dr Carty explained that recruitment would be from three streams, i.e. women who took part in the Generation Scotland Scottish Family Health Study and who had given permission to be contacted in the future, women from the Proteomics in Preeclampsia Study who had also attended postnatally, and also women who were attending the Blood Pressure Clinics in Glasgow Hospitals.

2) Dr Carty explained that ethical approval was required to contact women who took part in the Proteomics in Preeclampsia Study and who also attended postnatally. The Committee asked when the last contact had been with these women and how their current status would be checked. Dr Carty advised that it was a maximum of one/two years since the last contact. A check would be made through the CHI system to confirm their current status and their delivery details were also available as they had all been seen postnatally. The Committee was happy with this response.

3) In relation to recruitment from Blood Pressure Clinics, this would be done by the Research Nurse who would ask the Doctors and Nurses in the Clinics to tell the women about the study and if interested the Research Nurse they could approach the Research Nurse who would give them further information about the study.

   The Committee were in happy with the recruitment process.

4) The Committee noted that there would be a 10 year follow up. Dr Carty advised that there would be no actual contact with the women. They would only continue to collect data to establish whether these women go on to develop cardiovascular disease.

5) The Committee asked Dr Carty to explain what would happen if someone is identified during the study as having very high blood pressure. Dr Carty explained that in the first instance they would contact the GP but if it was considered to be very serious then the participant would be referred immediately to A&E. Dr Carty assured the Committee that time would be taken to explain the significance of the findings.

6) The Committee asked who would be responsible for the case note review to confirm the diagnosis of Preeclampsia. Dr Carty advised that the Lead Research Nurse would be responsible for going through case notes if they were available and to attempt to confirm the diagnosis of Preeclampsia. However some diagnosis could be vague.

7) The Committee asked why women who already had a diagnosis of cardiovascular disease were being excluded from the study. Dr Carty explained that the purpose of the study was to recruit women who had not yet had a diagnosis of cardiovascular disease as opposed to those who were already established.

Ethical review of research sites

NHS Sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

Additional Conditions specified by the REC

a) The 10 year follow up should be included in the Participant Information Sheet stating that this is a data collection exercise only and that no contact will be made. Consent should be given for this 10 year follow up so this should be included in the Consent Form.

b) The Participant Information Sheet should be revised as follows:

- The relevant logs and contact details should be at the beginning.
- There should be a clear statement about incidental findings and what action would be taken, i.e. if something serious is found then an immediate referral would be made.
- There should be details of an independent contact. This should be someone who knows about the study but not involved and can answer questions or give advice.
- There should be details of the NHS Complaints Procedure
- The words 'Thank you for reading this Information Sheet' should be inserted at the end.

c) It is suggested that perhaps consent should be taken to make future contact.

d) It is suggested that the date and version number of the Participant Information Sheet is left blank in the Consent Form as this could change over time.

It is responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

You should notify the REC in writing once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. The REC will acknowledge receipt and provide a final list of the approved documentation for the study, which can be made available to host organisations to facilitate their permission for the study. Failure to provide the final versions to the REC may cause delay in obtaining permissions.

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
GP/Consultant Information Sheets	1.1	06 November 2012
Investigator CV		
Letter of invitation to participant	GS.1.1	06 November 2012
Letter of invitation to participant	BP1.1	06 November 2012
Letter of invitation to participant	PIP 1.1	06 November 2012
Other: Letter of Thanks for Participating	1.1	06 November 2012
Participant Consent Form	1.1	06 November 2012
Participant Information Sheet: GS Women	1.1	06 November 2012
Participant Information Sheet: PIP Women	1.1	06 November 2012
Participant Information Sheet: BP Clinic	1.1	06 November 2012
Protocol	1.1	06 November 2012
REC application		20 November 2012

Membership of the Committee

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

12/WS/0306	Please quote this number on all correspondence

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee's best wishes for the success of this project.

Yours sincerely

Liz Jamieson

Liz Jamieson
Committee Co-ordinator
On behalf of Eoin MacGillivray, Vice Chair

Enclosures: List of names and professions of members who were present at the meeting and those who submitted written comments

"After ethical review – guidance for researchers"

Copy to: Dr David Carty
Dr Maureen Travers, NHS Greater Glasgow & Clyde

West of Scotland REC 3

Attendance at Committee meeting on 13 December 2012

Committee Members:

Name	*Profession*	*Present*	*Notes*
Dr Mark Fawcett	General Practitioner	Yes	
Dr Adam Burnel	Consultant Psychiatrist - Chair	No	
Mrs Bernadette Campbell	Primary Care Support Nurse	Yes	
Ms Susan Fleming	Public Health Researcher	Yes	
Dr Anja Guttinger	Consultant in Sexual & Reproductive Health	Yes	
Mrs Mary Keenaghan	Clinical Auditor	Yes	
Mr Eoin MacGillivray	Lay Member - Vice Chair	Yes	
Dr Angus McFadyen	Reader in Health Statistics	Yes	
Dr Stuart Milligan	Lecturer in Palliative and Cancer Care	Yes	
Dr Stephen Noble	Consultant Anaesthetist	Yes	
Mrs Gillian Notman	Joint Occupational Therapy Lead Advisor	No	
Mrs Helen Ross	Lay Member	Yes	
Mrs Rosie Rutherford	Lay Member	Yes	

Also in attendance:

Name	*Position (or reason for attending)*
Dr Judith Godden	Scientific Officer/Manager
Mrs Liz Jamieson	Committee Co-ordinator

Written comments received from:

Name	*Position*
Mrs Gillian Notman	Joint Occupational Therapy Lead Advisor

WoSRES
West of Scotland Research Ethics Service

NHS
Greater Glasgow and Clyde

West of Scotland REC 3
Ground Floor – The Tennent Institute
Western Infirmary
38 Church Street
Glasgow G11 6NT
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Dr Christian Delles
BHF Glasgow Cardiovascular Research Centre
126 University Place
Glasgow
G12 8TA

Date 17th January 2013
Your Ref
Our Ref
Direct line 0141 211 2123
Fax 0141 211 1847
E-mail Liz.Jamieson@ggc.scot.nhs.uk

Dear Dr Delles

Study title: **Cardiovascular consequences of preeclampsia study**
REC reference: **12/WS/0306**
Protocol number: **1.1**
IRAS project ID: **108706**

Thank you for your recent email. I can confirm the REC has received the documents listed below and that these comply with the approval conditions detailed in our letter dated 19 December 2012

Documents received

The documents received were as follows:

Document	*Version*	*Date*
Participant Consent Form	1.2	09 January 2013
Participant Information Sheet: BP Clinic	1.2	09 January 2013
Participant Information Sheet: GS Women	1.2	09 January 2013
Participant Information Sheet: PIP Women	1.2	09 January 2013

Approved documents

The final list of approved documentation for the study is therefore as follows:

Document	*Version*	*Date*
GP/Consultant Information Sheets	1.1	06 November 2012
Investigator CV		
Letter of invitation to participant	GS.1.1	06 November 2012
Letter of invitation to participant	BP1.1	06 November 2012

Letter of invitation to participant	PIP 1.1	06 November 2012
Other: Letter of Thanks for Participating	1.1	06 November 2012
Participant Consent Form	1.2	09 January 2013
Participant Information Sheet: BP Clinic	1.2	09 January 2013
Participant Information Sheet: GS Women	1.2	09 January 2013
Participant Information Sheet: PIP Women	1.2	09 January 2013
Protocol	1.1	06 November 2012
REC application		20 November 2012

You should ensure that the sponsor has a copy of the final documentation for the study. It is the sponsor's responsibility to ensure that the documentation is made available to R&D offices at all participating sites.

12/WS/0306 **Please quote this number on all correspondence**

Yours sincerely

Mrs Liz Jamieson
Committee Co-ordinator

Copy to: Dr David Carty, BHF Glasgow Cardiovascular Research Centre
Dr Maureen Travers, R&D – NHS Greater Glasgow and Clyde

Appendix 12: COPS NHS GG&C Board Management Approval

NHS
Greater Glasgow and Clyde

Coordinator: Dr Maureen Travers
Telephone Number: 0141 2116389
E-Mail: Maureen.travers@ggc.scot.nhs.uk
Website: www.nhsggc.org.uk/r&d

R&D Management Office
Western Infirmary
Tennent Institute
1st Floor 38 Church Street
Glasgow, G11 6NT,

28th January 2013

Dr Christian Delles
University of Glasgow
BHF Glasgow Cardiovascular Research Centre
126 University Place
Glasgow
G12 8TA

NHS GG&C Board Approval

Dear Dr C Delles

Study Title:	**Cardiovascular consequences of preeclampsia in women from the Generation Scotland: Scottish Family Health Study**
Principal Investigator:	**Dr C Delles**
GG&C HB site	**Western Infirmary**
Sponsor	**NHS Greater Glasgow and Clyde**
R&D reference:	**GN11CA468**
REC reference:	**12/WS/0306**
Protocol no: (including version and date)	**Version 1.1 (06/11/2012)**

I am pleased to confirm that Greater Glasgow & Clyde Health Board is now able to grant **Approval** for the above study.

Conditions of Approval

1. **For Clinical Trials** as defined by the Medicines for Human Use Clinical Trial Regulations, 2004
   a. During the life span of the study GGHB requires the following information relating to this site
      i. Notification of any potential serious breaches.
      ii. Notification of any regulatory inspections.

It is your responsibility to ensure that all staff involved in the study at this site have the appropriate GCP training according to the GGHB GCP policy (www.nhsggc.org.uk/content/default.asp?page=s1411), evidence of such training to be filed in the site file.

2. **For all studies** the following information is required during their lifespan.

NHS
Greater Glasgow and Clyde

a. Recruitment Numbers on a quarterly basis
b. Any change of staff named on the original SSI form
c. Any amendments – Substantial or Non Substantial
d. Notification of Trial/study end including final recruitment figures
e. Final Report & Copies of Publications/Abstracts

Please add this approval to your study file as this letter may be subject to audit and monitoring.

Your personal information will be held on a secure national web-based NHS database.

I wish you every success with this research study

Yours sincerely,

M Maureen Travers

Dr Maureen Travers
Research Co-ordinator

Cc: David Carty

Appendix 13: Privacy Access Committee approval form

Information Services Division

Area 151A
Gyle Square
1 South Gyle Crescent
Edinburgh, EH12 9EB
Telephone 0131 275 6000
Fax 0131 275 7606
www.isdscotland.org

NHS National Services Scotland

Dr C Delles
BHF Glasgow Cardiovascular Research Centre
Institute of Cardiovascular and Medical Science
126 University Place
GLASGOW
G12 8TA

Date 30 May 2013
Your Ref
Our Ref 103/12

Enquiries to Rachael Wood
Direct Line 0131 275 7028
Email rachaelwood@nhs.net

Dear Dr Delles

Cardiovascular consequences of preeclampsia

The Privacy Advisory Committee has considered and approved your application for a data linkage in support of the above study.

Conditions applied: None

Time period: As specified

Points highlighted: None

The approval of the Committee is for a period of 5 years from the date of this letter. Any change to the terms of your application, including changes in data user(s), additional data fields or extension of the time period approved must be requested through Susan Kerr, PAC Administrator on 0131 275 6445 or nss.pac@nhs.net

In order to progress your request please contact the eDRIS team on telephone 0131 275 7333 or email nss.eDRIS@nhs.net.

Please note that the following details about your application will be published under the following headings on the PAC website at http://www.nhsnss.org/pages/corporate/pac_meetings_and_decision_making.php later this year:

No	Title	Type	Summary	Date sent to PAC	PAC Responses	NSS Decision	Date Completed

If you have any queries about this please contact Patricia Ruddy patricia.ruddy@nhs.net.

Kind regards.

Yours sincerely

R Wood.

Dr Rachael Wood
Consultant in Public Health Medicine

cc eDRIS

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